# THE JOURNAL OF DAIRY RESEARCH

EDITED FOR

*The Committee of Management*

BY

A. T. R. MATTICK, B.Sc., Ph.D.
The National Institute for Research in Dairying, University of Reading

and

J. A. B. SMITH, Ph.D., D.Sc., F.R.I.C.
The Hannah Dairy Research Institute, Kirkhill, Ayr

ASSISTED IN THE SELECTION OF PAPERS FOR PUBLICATION BY

Dr L. BULL (Australia)
Prof. E. L. CROSSLEY (Reading)
Prof. D. B. CUTHBERTSON (Aberdeen)
Prof. I. de BURGH DALY, F.R.S. (Cambridge)
Dr H. P. DONALD (Edinburgh)
Sir PAUL FILDES, F.R.S. (London)
Prof. P. J. J. FOURIE (South Africa)
Prof. R. C. GARRY, F.R.S.E. (Glasgow)
Dr T. GIBSON (Edinburgh)
Dr J. HAMMOND, F.R.S. (Cambridge)
Dr J. O. IRWIN (London)
Dr A. G. LOCHHEAD, F.R.S. (C.) (Canada)
Prof. F. C. MINETT (Houghton)
Prof. W. RIDDET (New Zealand)
Dr K. C. SEN (India)
Sir JAMES A. SCOTT WATSON (London)

VOLUME 21

CAMBRIDGE
THE UNIVERSITY PRESS
1954

*Committee of Management*

PROF. H. D. KAY, C.B.E., F.R.S. (*Chairman*)
PROF. R. C. GARRY, F.R.S.E.
MR W. H. SENIOR
DR E. MARSDEN
SIR HERBERT HOWARD
DR A. T. R. MATTICK, DR J. A. B. SMITH } *Editors*
MR H. F. BURGESS, M.B.E. (*Secretary*)

---

*Librarian and Editorial Assistant*

MISS D. KNIGHT, B.A., F.L.A. Dip.Lib.

*Printed in Great Britain at the University Press, Cambridge*
*(Brooke Crutchley, University Printer)*
*and published by the Cambridge University Press*
*London: Bentley House, N.W.1*
*American Branch: New York*
*Agents for Canada, India and Pakistan: Macmillan*

98768

# CONTENTS

## No. 1 (February 1954)


ORIGINAL ARTICLES: PAGE

530. The relative feeding value for milk production of two National Cattle Foods. G. L. Bailey, W. H. Broster, B. B. Brown and A. S. Foot 1

531. The influence of live weight on milk production during the first lactation. G. L. Bailey and W. H. Broster . . . . . 5

532. The relationship between mastitis and the method of stripping after machine milking. F. K. Neave, T. M. Higgs, D. Simpkin, J. Oliver and F. H. Dodd . . . . . 10

533. Intramammary pressure changes in the lactating sow. I. The effect of oxytocin. W. G. Whittlestone . . . . . 19

534. The cells in bovine milk. P. S. Blackburn and Ian Macadam . 31

535. Reactivation of milk phosphatase following heat treatment. III. R. C. Wright and J. Tramer . . . . . 37

536. The physical properties of cow's milk as influenced by stage of lactation. The size distribution of butterfat globules. W. G. Whittlestone . . . . . 50

537. The composition of sow's milk during the course of lactation. Dawn R. Perrin . . . . . 55

538. The estimation of the energy of sow's milk from fat and total solids content. Dawn R. Perrin . . . . . 63

539. The effect of temperature treatment of cream before churning on the consistency of butter. R. M. Dolby . . . . . 67

540. A device for rapidly churning small quantities of cream. R. M. Dolby 78

541. A note on the vitamin D content of Indian butters. K. M. Henry and S. K. Kon . . . . . 81

542. The surface microflora of Stilton cheese—normal and slip-coat. Marjorie Brindley . . . . . 83

543. Methods for the growth and enumeration of anaerobic spore-formers from cheese with observations on the effect of nisin. A. Hirsch and Elisabeth Grinsted . . . . . 101

544. Factors influencing the lactic-acid producing properties of streptococci used in the manufacture of Cheddar cheese. I. Observations relating inhibitory and stimulatory phenomena. G. R. Jago . . 111

REVIEWS OF THE PROGRESS OF DAIRY SCIENCE:

Section C. Dairy chemistry and physics. Part. I. Chemistry and physical chemistry. R. Aschaffenburg and E. R. Ling. Part II. Physics (rheology). G. W. Scott Blair . . . . . 122

## No. 2 (June 1954)

Original Articles: PAGE

545. Studies of the secretion of milk of low fat content by cows on diets low in hay and high in concentrates. II. The effect of the protein content of concentrates. C. C. Balch, D. A. Balch, S. Bartlett, C. P. Cox, S. J. Rowland and Jill Turner . . . . . 165

546. Studies on the secretion of milk of low fat content by cows on diets low in hay and high in concentrates. III. The effect of variations in the amount and physical state of the hay and a comparison of the Shorthorn Friesian breeds. C. C. Balch, D. A. Balch, S. Bartlett, Zena D. Hosking, V. W. Johnson, S. J. Rowland and Jill Turner . 172

547. A recording tympanometer for the measurement of intramammary pressure in the cow. D. S. M. Phillips . . . . . 178

548. Intramammary pressure changes in the lactating sow. II. The effects of vasopressin and acetylcholine. W. G. Whittlestone . . . 183

549. Intramammary pressure changes in the lactating sow. III. The effects of level of dose of oxytocin and the influence of rate of injection. W. G. Whittlestone . . . . . 188

550. Colour changes in heated and unheated milk. I. The browning of milk on heating. H. Burton . . . . . 194

551. Variations in the fat content of human milk during suckling. W. G. Whittlestone and Dawn R. Perrin . . . . . 204

552. Heat-induced acidity in milk. F. H. Grimbleby . . . . 207

553. Moisture losses in Cheddar cheese undergoing curing. J. K. Scott . 212

554. Titration curves of whey constituents. M. Boulet and Dyson Rose 229

555. Aroma-producing group 'N' streptococci isolated from Cheddar cheese curd. M. Zielinska and E. R. Hiscox . . . . . 238

Reviews of the Progress of Dairy Science:

Section D. Nutritive value of milk and milk products. S. K. Kon and K. M. Henry . . . . . 245

## No. 3 (October 1954)

Original Articles:

556. The use of seaweed meals in concentrate mixtures for dairy cows. A. W. A. Burt, S. Bartlett and S. J. Rowland . . . . 299

557. Studies in the secretion of milk of low fat content by cows on diets low in hay and high in concentrates. IV. The effect of variations in the intake of digestible nutrients. C. C. Balch, D. A. Balch, S. Bartlett, V. W. Johnson, S. J. Rowland and Jill Turner . . . . 305

558. The secretion of a single tracer dose of labelled iodide in the milk of the lactating cow. R. F. Glascock . . . . . 318

559. The inhibition of micro-organisms by raw milk. III. Distribution and properties of two inhibitory substances, lactenin 1 and lactenin 2. J. E. Auclair . . . . . 323

ORIGINAL ARTICLES PAGE

560. The incidence of penicillin in milk supplies. F. C. STORRS and WINIFRED HIETT-BROWN . . . . . 337

561. The use of phage typing and penicillin sensitivity tests in studies of staphylococci from bovine mastitis. PATRICIA PRICE, F. K. NEAVE, JOAN E. RIPPON and R. E. O. WILLIAMS . . . . . 342

562. The steam stripping of taints from liquids. I. An analysis of processing methods with particular reference to the Vacreator. J. K. SCOTT . . . . . 354

563. The steam stripping of taints from liquids. II. Counterflow stripping with particular reference to possible use of Vacreator equipment. J. K. SCOTT . . . . . 370

564. The properties of New Zealand butters and butterfats. IV. Refractive index and density of the butterfat. A. K. R. MCDOWELL . . . 383

REVIEWS OF THE PROGRESS OF DAIRY SCIENCE:

Section E. Diseases of dairy cattle. P. S. BLACKBURN . . . 391

Section A. Physiology of dairy cattle. E. C. OWEN . . . . 408

THE JOURNAL OF THE

# SOCIETY OF DAIRY TECHNOLOGY

17 DEVONSHIRE STREET, LONDON, W.1. *Tel. Langham* 5059

The Journal is issued in quarterly parts, four parts constituting a volume. The parts are usually issued in January, April, July and October each year.

The Subscription rate to non-members of the Society, payable in advance, is £2 net per volume (post free): single numbers, 12*s.* 6*d.* net. Subscriptions and all business enquiries should be sent to the Secretary, Society of Dairy Technology, 17 Devonshire Street, London, W.1.

Each member of the Society receives one free copy of each number. One further copy of each number may (if available) be bought by members for 7*s.* 6*d.*

CONTENTS OF THE JANUARY ISSUE

Tenth Annual General Meeting.
Further Aspects of the New Zealand Dairy Industry, by Professor W. RIDDET.
The Technology of Milk Condensing and Drying, by W. P. CROCKER.
Sectional Notes.
Dairying History No. 3: Some Early Recollections of Dairying in the South of England, by W. R. B. ST JOHN GATES.
Book Reviews.
Factors in the Progress of Dairying in a Welsh County (Carmarthenshire), by JOHN LEWIS.
Council and Officers 1953/4. List of Members.

*The Journal publishes not only papers read at meetings of the Society, but also papers on dairy technology by members and non-members*

*Advertising Offices:* 9 GOUGH SQUARE · FLEET STREET · E.C.4

## Indian Journal of Dairy Science

Established in 1948 as a medium for the advancement and dissemination of knowledge in regard to dairy science. Published quarterly in March, June, September and December. Subscription £1 sterling or $4.00 in dollar areas. For further particulars apply to the Hon. Joint Secretaries, Indian Dairy Science Association, Hosur Road, Bangalore 1 (India).

Editor: K. C. Sen, D.Sc., F.N.I.

CONTENTS OF VOL. VI, No. 4, DECEMBER 1953

*T. Narayanan and K. K. Iya. Studies on Bovine Mastitis. 1. Incidence of Mastitis in Cows and Buffaloes.*

*M. T. Ragab, A. A. Asker and M. S. Ghazy. Effect of Age on Total Milk Yield and Length of Lactation Period in the Egyptian Buffalo.*

*K. M. Narayanan, C. P. Anantakrishnan and K. C. Sen. Vitamin A in Dairy Products. Part 1. Stability of Vitamin A and Carotene in Ghee Fortified with Synthetic Vitamin A.*

*I. N. Mahmoud. Bacterial Contaminants in Buffalo Semen.*

*K. C. Sen, G. K. Murthy, B. N. Premachandra, Noshir N. Dastur and S. C. Ray. Effect of Feeding Iodinated Casein on Growth and Food Utilization by Calves.*

*A. M. El-Sokkary and M. H. Zaki. Stability of Buffalo, Cow, Sheep and Goat Samna (Ghee) against Oxidative Deterioration.*

*K. C. Sen, A. J. Lazarus, M. C. Rangaswamy and C. P. Anantakrishnan. Observations on Some Indian Cattle. Part V. Cross-Breeding for Milk Production.*

## COMMONWEALTH BUREAU OF DAIRY SCIENCE

SHINFIELD, READING, ENGLAND

The functions of the Bureau are to collect, abstract, collate, review and distribute information on everything concerned with research and new developments in dairy science. It is in fact a clearing house of world information on all matters concerned with dairy husbandry, technology and science, covering the milk of all mammals, including human milk, and dealing with the many dairy processes and products.

# DAIRY SCIENCE ABSTRACTS

COMPILED FROM WORLD LITERATURE

is prepared every month from the world's dairying literature. This monthly journal—the only one of its kind in the world—contains abstracts of all new and important work reported in the various fields of dairying as well as review articles on specialized subjects.

All inquiries, editorial and technical matters, should be addressed to the Director at Shinfield, and all subscriptions to the Central Sales Branch at Farnham Royal.

*Subscription rates: foreign* — *50s. per volume*

*U.K. and British Commonwealth*
(for orders sent direct, other than trade) — *40s. per volume*

COMMONWEALTH AGRICULTURAL BUREAUX

CENTRAL SALES BRANCH, FARNHAM ROYAL, SLOUGH, ENGLAND



# The Journal of General Microbiology

Editors: B. C. J. G. KNIGHT and A. F. B. STANDFAST


*Contents of Vol. 10, No. 1*


**Rose, R. E. and Miller, J. G.** Some sampling variations in soil fungal numbers.
**Rozansky, R., Bachrach, U. and Grossowicz, N.** Studies on the antibacterial action of spermine.
**Fothergill, P. G. and Raine, L. C. D. P.** The mineral nutritional requirements of *Mucor Hiemalis* Wehm.
**Edward, ff. D. G.** The pleuropneumonia group of organisms: a Review, together with some new observations.
**di Menna, M. E.** *Debaryomyces marama* n.sp., isolated from the air.
**Leifson, E. and Hugh, R.** A new type of polar monotrichous flagellation.
**Buxton, E. W.** Heterocaryosis and variability in *Fusarium oxysporum* F. *gladioli* (Snyder & Hansen).
**Saperstein, S., Starr, M. P. and Filfus, J. A.** Alterations in carotenoid synthesis accompanying mutation in *Corynebacterium michiganense*.
**Lewin, R. A.** A marine stichococcus sp. which requires vitamin $B_{12}$ (Cobalamin).
**Tomcsik, J. and Guex-Holzer, S.** Demonstration of the bacterial capsule by means of a pH-dependent, salt-like combination with proteins.
**Sussman, M.** Synergistic and antagonistic interactions between morphogenetically deficient variations of the slime mould, *Dictyostelium discoideum*.
**Jennens, M. G.** The methyl red test in peptone media.
**Jennens, M. G.** The effect of desiccation on antigenic structure.
**Mager, J., Kindler, S. H. and Grossowicz, N.** Nutritional studies with *Clostridium parabotulinum* type A.
**Mann, S. O., Masson, F. M. and Oxford, A. E.** Facultative anaerobic bacteria from the sheep's rumen.
**D'Arcy Hart, P. and Rees, R. J. W.** Changes in morphology of an avirulent strain of *Mycobacterium tuberculosis* under the influence of non-ionic surface-active agents.
**Bawden, F. C. and Kassanis, B.** Some effects of thiouracil on virus-infected plants.
**Cowan, S. T., Shaw, C. and Williams, R. E. O.** Type strain for *Staphylococcus aureus* Rosenbach.
**Painter, H. A.** Factors affecting the growth of some fungi associated with sewage purification.



*The subscription rate is 90s. net per volume of 3 parts, Single parts 35s. each, plus postage.*
*Two volumes will be issued each year. Orders should be sent to*

**CAMBRIDGE UNIVERSITY PRESS**

**BENTLEY HOUSE, 200 EUSTON ROAD, LONDON, N.W.1**

636.2.085

# 530. THE RELATIVE FEEDING VALUE FOR MILK PRODUCTION OF TWO NATIONAL CATTLE FOODS

BY G. L. BAILEY, W. H. BROSTER, B. B. BROWN AND A. S. FOOT
*National Institute for Research in Dairying, University of Reading*

The feeding value of artificially dried forage crops may vary from that of air-cured hay to a value comparable with that of the medium protein oil-seed residues. This wide variation makes it very difficult for the farmer to integrate these foods economically into a general rationing scheme. It is true that the crude protein content of dried grass is a fair measure of its feeding value and this figure is usually available, but with crops such as lucerne the same standards cannot be used, and much experience is necessary before its value can be estimated with accuracy. To overcome these difficulties, formulae have been devised to enable artificially dried forage crops of high protein content to be compounded with cereals into cattle cubes of a feeding value comparable with National Cattle Food No. 1. The cubes so compounded were known as National Cattle Food No. 6 (1). The main source of the protein in N.C.F. 1 was imported oil-seed residues, whereas the protein in N.C.F. 6 came predominantly from home-grown artificially dried forage crops.

This paper consists of a report on the experimental method and the results of a field trial of the relative feeding value for milk production of the two foods.

## METHOD

The trial was carried out during the indoor feeding period of the winters of 1951/2 and 1952/3. The animals used were all first calvers. In the first season there were nine Friesians, eleven Shorthorns, one Guernsey and one Guernsey × Shorthorn. In the second season five of the experimental animals were Friesians, fourteen were Shorthorns and again there was only one Guernsey. The animals were machine milked in a milking parlour where they received a small quantity of their concentrate rations; the balance of their rations was given them when they were tied up in covered yards. They spent the nights in these yards, but during the daytime they were turned out on to bare pasture for exercise.

The animals were fed rations consisting of hay and a mixture of N.C.F. 1 and N.C.F. 6. Different batches of these foods were used in the two seasons, but enough of each food was obtained at the beginning of each season to last throughout that season. The chemical analyses of these foods and the manufacturer's formulae for the No. 6 cubes are given in Table 1.

Two systems for determining the quantities of the foods to be fed were used; in the first season the starch equivalent requirements for each animal were determined from the milk yield. Then the proportion of the total to be met by each of the three foods was decided by chance. This system was considered to be unsatisfactory owing to the necessity of judging the relative value of the foods before the results of the trial were known, and the impossibility of permitting the quantity of hay fed to vary at random; for this food substantially controlled the bulk of the ration, and this necessarily fluctuated within fairly

Table 1. *Chemical analysis of foods used*

| Part of experiment | Food | Dry matter (%) | Analysis of dry matter by percentage: Crude Protein | Ether extract | Soluble carbo-hydrates | Fibre | Ash |
|---|---|---|---|---|---|---|---|
| Winter 1951/2 | Hay | 89 | 15·2 | 1·8 | 43·4 | 31·1 | 8·6 |
| | N.C.F. 1 | 92 | 19·0 | 4·9 | 59·4 | 9·4 | 7·3 |
| | N.C.F. 6 | 92 | 20·4 | 2·5 | 47·0 | 19·7 | 10·4 |
| Winter 1952/3 | Hay | 84·3 | 10·4 | 1·6 | 44·4 | 36·3 | 7·4 |
| | N.C.F. 1 | 85·3 | 21·4 | 3·5 | 56·8 | 10·6 | 7·7 |
| | N.C.F. 6 | 88·2 | 20·3 | 3·1 | 47·8 | 19·1 | 9·7 |

*Manufacturer's formula for N.C.F. 6*

| Winter 1951/2 | Winter 1952/3 |
|---|---|
| Lucerne 50% | Lucerne 73·5% |
| Grass 25% | Barley 5% |
| Barley 9·4% | Maize 5% |
| Maize 9·35% | Sorghum 5% |
| Bran 6·25% | Low-protein oilcake 10% |
| | Minerals 1% |
| | Salt ½% |

close limits. In the second season a basic ration of hay varying at random from 6 to 20 lb. was allocated; 16 lb. of this hay was judged adequate for maintenance and 2 lb. of it was judged equal to 1 lb. concentrates. The surplus requirements were made up by one of three possible mixtures of N.C.F. 1 and N.C.F. 6. The mixtures were made up in three proportions 2:1, 1:1, 1:2. All these mixtures were fed at the rate of 4 lb. to 10 lb. milk. The allocation of a particular mixture to a particular cow was at random. The mean consumption of the foods in each of the years is given in Table 2.

Table 2. *Food Consumption*

| | Mean hay (lb.) | Mean N.C.F. 1 (lb.) | Mean N.C.F. 6 (lb.) | Mean Live weight (lb.) |
|---|---|---|---|---|
| 1951/2 | 12·75 | 9·45 | 9·70 | 1070 |
| 1952/3 | 12·80 | 8·52 | 7·57 | 1069 |

The foods offered and any refused were weighed daily. The yield of milk was recorded at each milking. In the first season the animals were weighed once a month and in the second season twice a week.

The trial was planned to take place at a predetermined stage in the animal's lactations rather than at a particular calendar time. In the first season the animals were placed on the experimental rations 6 weeks after calving, and the test period was 4 weeks later. Throughout this time the rations were adjusted weekly according to the milk yield in the previous week. In the second season the animals were given the experimental rations from immediately after calving and the test period was 6 weeks later. While the milk yields of the animals were increasing, the rations were adjusted weekly according to the yield anticipated for the following week. As soon as the peak yield was reached, and in all cases before the sixth week, the rations were adjusted to the yield in the previous week. The exact timing of the test period could not in practice be achieved for all the animals. This was due to the late grazing which was available in the autumn months of both seasons. To benefit from this the animals were kept out at pasture longer than was

anticipated, and some of the animals were at a later stage of lactation than was intended by the time they were settled on the indoor feeding. In the first season at the time of the test period the average time in milk was 94 days instead of the planned 70 days, and their average daily milk yield was 30½ lb; in the second season the time was 49 days instead of the planned 42 days, and the average daily milk yield was 28¼ lb.

The test period in each season lasted 1 week, and the data collected during this time were used to calculate daily means. These mean figures were used to construct for each animal an input/output relationship of the form

$$\text{Milk yield (lb.)} = \text{hay (lb.)} \times b_1 + \text{N.C.F. 1 (lb.)} \times b_2 + \text{N.C.F. 6 (lb.)} \times b_3 - \text{live weight}^{\frac{3}{4}} \text{ (lb.)} \times b_4,$$

where $b_1$, $b_2$, $b_3$, $b_4$ are coefficients of unknown value. The values of the $b$'s which gave the best fit of the data from all the animals used in each year were calculated by the normal methods for multiple regression. They are given in Table 3.

Table 3. *Calculated values of the 'b' coefficients*

| | Hay | N.C.F. 1 | N.C.F. 6 | Live weight$^{\frac{3}{4}}$ | Ratio N.C.F. 1 / N.C.F. 6 |
|---|---|---|---|---|---|
| First season | 0·639 ± 0·531 | 1·797 ± 0·396 | 1·767 ± 0·294 | −0·143 ± 0·153 | 1:1·017 |
| Second season | 0·317 ± 0·335 | 1·575 ± 0·292 | 1·466 ± 0·436 | 0·0496 ± 0·165 | 1:1·075 |

## RESULTS

The difference between the two foods was very small in the first year of the trial. The coefficient for N.C.F. 1 was 1·797 and that for N.C.F. 6 was 1·767. The difference between these was statistically not significant, being 0·030 ± 0·274; however, the ratio between the two, 1 : 1·017, showed the possibility of a small advantage to the N.C.F. 1. In this first year the animals' weights were determined once a month only and as a result there was little check on the changes in live weight. The rate of live-weight gain was not uniform for all the animals so the results may be biassed in some way. For this reason it is thought that the results of the second year of the trial are more dependable, for the animals were weighed twice a week which permitted a check on their rate of growth; this was made and showed a good measure of uniformity between the animals. The difference between the coefficients was 0·109 ± 0·2313 again without statistical significance, but the ratio between the N.C.F. 1 and N.C.F. 6, 1 : 1·075, again showed an advantage to the N.C.F. 1. It should be noted that if the coefficients were corrected for the different dry-matter content of the cubes, the ratio would be increased. The values of the coefficients N.C.F. 1 = 1·575 and N.C.F. 6 1·466 were somewhat lower than in the first year. They reflect the differences in the chemical analyses of the foods in the two seasons (Table 1), but it is doubtful if this source of difference was sufficient to account fully for the differences in the magnitude of the coefficients. It appears probable that the main source is the difference in the mean milk yields of the animals in the two years, and possibly the change in the proportions of the breeds.

## DISCUSSION

The results of the feeding trials indicate that the standards for the compounding of N.C.F. 1 and N.C.F. 6 produce foods of very similar feeding value. Though there may be a tendency for the N.C.F. 1 to produce rather more milk than the N.C.F. 6 the extent of

the difference would be hardly noticeable under normal commercial conditions. The difference found in this trial was only 0·109 lb. milk from each 1 lb. of cubes fed; thus the difference in yield of milk if 9 lb. of N.C.F. 1 were given instead of 9 lb. of N.C.F. 6 would be a gain of not quite 1 lb. This trial was carried out with animals of only moderate yield, and it should not be assumed from the results that the two foods would be of equal value to very high-yielding cows. The high fibre content of the no. 6 cubes might reduce their value when used in the large quantities necessary for such animals.

This study is of interest not only for the comparison between the two foods, but also as an illustration of a little-used experimental method. The following two advantages are claimed for the method: (1) A critical experiment may be conducted with a group of animals of so mixed characteristics that it would be exceedingly difficult to obtain from them fairly paired or blocked animals suitable for an experiment of classic design; the advantage arises from the test materials being compared within rather than between animals. (2) In the event of one or more animals partly refusing their rations, the interpretation of the results of an experiment of classic design becomes difficult; the design illustrated here can cope with such a situation without any difficulty or complication.

Both these advantages are purely practical in that they make the experiment easier to conduct. Against them must be set the additional labour involved in the statistical analysis and the drawback that the results of the trial cannot be simply demonstrated but depend on the analyses.

SUMMARY

A feeding trial of the relative value for milk production of National Cattle Food No. 1 and National Cattle Food No. 6 is reported; forty-two first calvers were employed.

The two foods were shown to be of very similar feeding value for heifers giving two to four gallons daily, the difference between them being slightly in favour of the N.C.F. 1. Each pound of this food produced 0·109 lb. more milk than the same amount of N.C.F. 6, but this difference was statistically without significance.

REFERENCE

(1) Statutory Instrument 1951, no. 1135.

(*MS. received for publication* 12 *August* 1953)

637.12:612.664.36

# 531. THE INFLUENCE OF LIVE WEIGHT ON MILK PRODUCTION DURING THE FIRST LACTATION

By G. L. BAILEY and W. H. BROSTER

*National Institute for Research in Dairying, University of Reading*

The existence of a positive relationship between the live weight of a dairy cow and her milk production has been demonstrated by many workers (1, 2, 3). If the relationship applies generally, the average milk yield of a herd could be increased by culling the small animals. In the long term the maintenance of the higher level of production by the daughters of the larger animals would depend on the relative importance of size to other factors of production associated with the use of a particular bull and on the extent to which size is inherited. The study reported here was planned to test these theories.

## METHOD

This study was made with the records collected from first lactations occurring during the years 1942–52 in the Dairy Shorthorn herd maintained at Shinfield by the National Institute for Research in Dairying. Particulars of the management and breeeding of this herd have been described in some detail in an earlier paper (4); it is sufficient here to note that it is not the policy to select animals for their size or to adjust the rations either according to their size or milk-fat production.

The records of all the first lactations were arranged in groups according to the sire of the animals producing the records; groups consisting of less than five animals were discarded. It was found that records of ninety-nine animals sired by seven different bulls were available for study. The records tabulated were: (1) live weight at calving (lb.); (2) age at calving (days); (3) lactation length (days); (4) lactation yield of 4% fat-corrected milk (lb./10) (see ref. (5)). The animals were weighed within 7 days of calving on a weighbridge capable of accurate measurement within 1 lb. In a very few cases the weighing was delayed for a few days after the 7-day period. Records of these single weighings for each animal were used in the study. The age at calving was calculated in days for each animal from the recorded dates of birth and first calving. This variable was included in order to examine the effect of variations in age at calving on the subsequent production of milk. Furthermore, the treatment of the data is made directly comparable to that practised by Gaines (1, 2, 6). The lactation length was obtained from the recorded dates of the start and finish of milk recording for the lactation. Milk recording began 4 days after the date of calving. Although the lactation length was not used by Gaines (1, 2, 6), it was included here in order to afford a statistical control of error. The lactation yield of 4% fat-corrected milk was calculated from the lactation yield of milk and the lactation yield of milk fat estimated from the monthly tests of fat percentage (see (4) for details of the test technique). The figure was calculated for each cow in units of lb./10 in order to simplify the calculations. The mean values of these data are given in Table 1.

Table 1

| Bull | No. of daughters | Mean live weight at calving (lb.) | Mean age at calving (days) | Mean lactation length (days) | Mean lactation yield of 4% F.C.M. |
|---|---|---|---|---|---|
| A | 27 | 1035 | 1092 | 318 | 650 |
| B | 20 | 1000 | 1061 | 298 | 630 |
| C | 15 | 1003 | 1066 | 301 | 691 |
| D | 12 | 1065 | 1077 | 282 | 594 |
| E | 11 | 1014 | 1129 | 338 | 622 |
| F | 8 | 1137 | 1074 | 280 | 631 |
| G | 6 | 963 | 1056 | 371 | 643 |
| Total | 99 | 1028 | 1081 | 309 | 640 |

The relationship between an animal's live weight and her production of milk can be expressed mathematically in the form

$$FCM = cLW^b,$$

where $FCM$ = production of milk, $LW$ = live weight, $c$ = a constant, $b$ = exponent of $LW$. Kleiber (7, 8, 9) and Brody (10) have advanced theoretical reasons and experimental evidence for the value of $b = 0{\cdot}75$, but there is considerable variation in the value when determined for different experimental data (2, 10, 11). When the $FCM$ was measured as the production during the first 305 days of the lactation, the data for the cows studied here produced the function

$$FCM_{305} = 0{\cdot}6195LW^{0{\cdot}8207 \pm 0{\cdot}2159}.$$

The difference between this experimentally determined value of $b$ and the value generally accepted is quite small. As neither of these functions departs very markedly from linearity over the range of values of live weight actually used in this study, it was decided that a linear function would adequately express the relationship. Multiple linear regression equations were calculated, using the lactation yield of 4% F.C.M. as the dependent variate and live weight, age at first calving and lactation length as the independent variates. Equations were calculated both from the entire data and for the seven groups each representing the progeny of one bull. The homogeneity of the variances of these groups was tested (Bartlett (12)), and so were the significances of the differences between the mean within bull-group regression and the individual bull group regressions. The results of these calculations are given in Tables 2 and 3. The extent to which size is inherited was measured by the calculation of a correlation coefficient; it was found that the data provided

Table 2. *Partial regression coefficients*

| Bull | $b_{y1.23}$ | $b_{y2.13}$ | $b_{y3.12}$ |
|---|---|---|---|
| A | $0{\cdot}4036 \pm 0{\cdot}1584$ | $-0{\cdot}0020 \pm 0{\cdot}1724$ | $1{\cdot}7910 \pm 0{\cdot}3380$ |
| B | $0{\cdot}2000 \pm 0{\cdot}2045$ | $0{\cdot}0741 \pm 0{\cdot}3374$ | $2{\cdot}141 \pm 0{\cdot}4297$ |
| C | $0{\cdot}2828 \pm 0{\cdot}4936$ | $-0{\cdot}4917 \pm 0{\cdot}5134$ | $2{\cdot}8025 \pm 0{\cdot}5972$ |
| D | $0{\cdot}9620 \pm 0{\cdot}5804$ | $0{\cdot}2796 \pm 0{\cdot}6634$ | $1{\cdot}4208 \pm 0{\cdot}8078$ |
| E | $0{\cdot}8249 \pm 0{\cdot}4048$ | $-0{\cdot}2804 \pm 0{\cdot}2507$ | $2{\cdot}593 \pm 0{\cdot}6934$ |
| F | $0{\cdot}2788 \pm 0{\cdot}6672$ | $0{\cdot}9425 \pm 1{\cdot}6440$ | $-0{\cdot}8913 \pm 4{\cdot}8870$ |
| G | $0{\cdot}9825 \pm 0{\cdot}4224$ | $0{\cdot}0619 \pm 0{\cdot}4221$ | $1{\cdot}6715 \pm 0{\cdot}7649$ |
| Total data | $0{\cdot}5141 \pm 0{\cdot}1052$ | $-0{\cdot}1120 \pm 0{\cdot}1168$ | $1{\cdot}9102 \pm 0{\cdot}1900$ |
| Between bulls | $-0{\cdot}1787 \pm 0{\cdot}5006$ | $-0{\cdot}2530 \pm 0{\cdot}8426$ | $0{\cdot}1022 \pm 0{\cdot}9411$ |
| Within bulls | $0{\cdot}5044 \pm 0{\cdot}1264$ | $-0{\cdot}0780 \pm 0{\cdot}1313$ | $1{\cdot}4890 \pm 0{\cdot}2283$ |

$y$ = lactation yield of 4% fat-corrected milk.
1 = live weight, 2 = age, 3 = lactation length.

information on live weight at calving of forty-seven dam/daughter pairs, the daughters being sired by six different bulls. The correlation coefficient between these weights was calculated.

Table 3. *Analyses of variance*

| Source | D.F. | S.S. | M.S. | F |
|---|---|---|---|---|
| Total | 98 | 2,732,366 | | |
| Due to regression | 3 | 1,578,477 | 526,159 | 43·320** |
| From regression | 95 | 1,153,889 | 12,146 | |
| Between bulls | 6 | 73,540 | | |
| Due to regression | 3 | 11,091 | 3,697 | Non-sig. |
| From regression | 3 | 62,449 | 20,816 | |
| Within bulls | 92 | 2,658,826 | | |
| Due to regression | 3 | 1,299,183 | 433,061 | 29·304** |
| From regression (mean) | 89 | 1,359,643 | 14,778 | |
| From regression (individual group regressions) | 71 | 927,809 | 13,067 | |
| Differences between mean regression and individual group regressions | 18 | 431,834 | 23,991 | 1·836* |

* Significant when $P=5\%$. ** Significant when $P=1\%$.

## RESULTS

The partial regression coefficients given in Table 2 exhibit some variation, and the test made in the analysis of variance on the differences between the mean regression and the individual bull-group regressions showed that these differences were just significant when $P=5\%$. That the entire data was not homogeneous was further shown by the high value of $\chi^2=17{\cdot}48$ (when $P=1\%$, $\chi^2=16{\cdot}81$). These tests showed that the daughters of one bull are more alike in the variables studied than a group of cows of mixed inheritance, or, stated in another way, a group of cows from one sire exhibit characteristics peculiar to itself which are not shared by other groups.

In spite of the evidence that the entire data represented a heterogeneous population, certain features common to all the groups were apparent in the analysis. Thus in all cases the yields increased with the live weights and with the lactation lengths but were unaffected by ages at calving. There was one very important exception to this general finding. The regression between the means of bull-groups did not depend on either live weight, lactation length or age. This result showed that the groups of cows from one bull which produced the higher mean yields were not those that had the higher mean live weights.

The coefficient calculated for the correlation between the live weights at calving of dams and daughters was statistically non-significant ($r=0{\cdot}2356$; when $P=5\%$, $r=0{\cdot}3044$, D.F. 40). It may well be that with a larger number of animals, significance would be obtained, but even so it is clear that the correlation is low, and that the live weight of the dam at first calving has little relation to the live weight of the daughters at first calving.

## DISCUSSION

The results of this study support the general finding that the yield of 4% F.C.M. varies directly with the live weight at calving. However, the design of the study demonstrated an important exception to the applicability of the relationship. The mean yields of groups of cows, each group sired by one bull, can be quite independent of their mean live weights. This is clearly shown by the non-significant value of the between bull-groups regression

found in this study. This suggests that the mean yield of a bull group is determined more by the qualities of the sire than by the group's mean live weight. The value found here of course applies to this study only. However, if the qualities other than live weight of the bull are the overruling considerations, then it is logical to conclude that the value of the between bull-group regression will vary according to the particular population studied. It may well be possible to find herds in which by chance the regressions might be of any value or any statistical significance.

The bull-group is the unit by which any breeding policy within a herd aims to improve the mean production of the herd. The results of the study show that the mean production of this unit will not be improved, except by chance, by giving attention to the live weight of the animals.

The validity of this claim is supported by the finding that the correlation coefficient calculated for the live weights at calving of dams and daughters is low, which shows that within the framework of an orthodox herd breeding policy further selection for high live weight will have little effect on the live weight of the next generation. Therefore, within a herd, variations in size would appear to be due primarily to chance differences in environmental conditions, and that these, if favourable, will produce large animals. Animals so produced will, on average, be the high yielders of the herd, but they will be unable to transmit to their daughters either their relatively great size or that part of their capacity to produce high milk yields which is associated with their size.

The characteristics of a population of cows wherein the positive relationship between the live weight of the cows and their milk production can be expected to exist, may now be outlined. The population must be fully representative of any entire breed or strain within breed. If the population consists of a selection within either of these units the relationship can operate only by chance. An example of such a population could be found by selecting the daughters of only two bulls of widely different production potential. If the high-yielding group happened to be small animals and the low yielders large, the relationship might not apply to the combined groups, yet there would be a high probability that it would apply within each group separately.

SUMMARY

The relationship between live weight and lactation production was examined; records from ninety-nine animals, daughters of seven bulls, were used.

A positive relationship was found to exist within each of the seven groups and within the entire population.

The relationship did not exist between the mean values of the group.

No correlation was found between the live weights at calving of forty-seven dam/daughter pairs; the daughters were the offspring of six bulls.

It was shown that a herd breeding policy which selected animals according to their size would fail, except by chance, to improve the production of the herd.

## REFERENCES

(1) Davis, H. P., Morgan, R. F. & Gaines, W. L. (1943), *J. Dairy Sci.* **26**, 625.
(2) Gaines, W. L. (1941). *J. Dairy Sci.* **24**, 795.
(3) Turner, C. W., Ragsdale, A. C. & Brody, S. (1924). *Bull. Mo. Agric. Exp. Sta.* no. 221.
(4) Bailey, G. L. (1952). *J. Dairy Res.* **19**, 89.
(5) Gaines, W. L. (1928). *Bull. Ill. agric. Exp. Sta.* 308.
(6) Gaines, W. L. (1947). *J. Dairy Sci.* **30**, 273.
(7) Kleiber, M. & Mead, S. W. (1945). *J. Dairy Sci.* **28**, 49.
(8) Kleiber, M. & Mead, S. W. (1941). *J. Dairy Sci.* **24**, 127.
(9) Kleiber, M. (1947). *Physiol. Rev.* **27**, 4.
(10) Brody, S. (1942). *Science*, **95**, 485.
(11) Gaines, W. L. (1946). *J. Dairy Sci.* **29**, 259.
(12) Bartlett, M. S. (1937). *J. R. statist. Soc.* **4**, 137.

(*MS. received for publication* 9 *July* 1953)

619.2:616.99965:6

# 532. THE RELATIONSHIP BETWEEN MASTITIS AND T... METHOD OF STRIPPING AFTER MACHINE MILKING

By F. K. NEAVE, T. M. HIGGS, D. SIMPKIN
*Bacteriology Department*
J. OLIVER and F. H. DODD
*Dairy Husbandry Department*
*National Institute for Research in Dairying, University of Reading*

At the end of normal machine milking some milk, which cannot be removed unaided even by the most efficient milking machine, remains in the udder sinus. This milk, often some 10% of the total yield depending upon the age of the cow, udder shape, partial vacuum of the milking machine and teat-cup liner design, was formerly most frequently removed by hand. As a result of advice and encouragement farmers are rapidly changing to manipulation of the teat-cups, known as machine stripping. This practice has renewed interest in the old controversy about the effect of the method of stripping on udder health.

The reported results of investigations in this field show little agreement. The evidence of early workers such as Ernst, Schmidt-Hoensdorf & Schmidt (1), Schmidt-Hoensdorf & Schmidt (2) and Schalm & Mead (3) was that the omission of stripping is followed by an increase in clinical mastitis. On the other hand, the surveys of Cullity (4) and Nichols (5) show a decrease in the amount of udder troubles when hand stripping is discontinued, while the more recent observations of Ward (6) and Ward & Castle (7) indicate that the incidence of mastitis is not affected by the method of stripping. In a related field Edwards (8) and Wilson (9) have shown that it is more difficult to eradicate mastitis caused by *Streptococcus agalactiae* in hand-stripped herds.

It is reasonable to suppose that omission of hand stripping and the adoption of machine stripping would reduce the spread of the common udder pathogens and so reduce mastitis. To test this in practice and to observe the effect of omitting the use of a strip-cup, investigations were carried out for 12 months starting in 1951.

## HERDS USED

Two herds of British Friesian cattle were used, both milked in conventional cowsheds with bucket-type milking machines. Herd Cr was milked at a partial vacuum of about 15·6 in. Hg with straight extruded teat-cup liners and herd Ba at a vacuum of about 15·3 in. Hg with moulded liners.

In herd Cr, where about thirty-eight cows were milked, there was little mastitis, and at the start of the experiment only four cows were found infected with *Str. agalactiae*, whereas in herd Ba about 87% of the thirty cows in milk were infected. The latter herd had been founded with surplus mature cows from another farm.

## METHODS

In both herds the animals were divided into two groups, on the basis of age, stage of lactation and udder infection. The two groups, one stripped by hand and the other by machine, were housed in separate sheds, and newly calved animals were, as far as

...able, allocated to keep the number of old and young animals in each group ...imately equal.

The farmers agreed to wash all udders with a hypochlorite solution (about 800 p.p.m. available chlorine) before milking. Two udder cloths were used alternately, and the solution of hypochlorite changed after washing nine cows. No attempt was made to disinfect the teat-cups before milking each cow.

In herd Cr, a strip-cup was used immediately after washing individual udders, while on farm Ba, which did not use a strip-cup, all udders were washed before milking the first cow. Small teat sores, which were particularly prevalent in herd Ba, were treated with proflavin cream (B.P.C.). Superficial disinfection of the hands, before stripping each cow, was practised in herd Ba only.

Two cows in each group were left infected with *Str. agalactiae* to act as a source of the organism. All the remainder in herd Ba were treated with penicillin in all quarters, and new *Str. agalactiae* infections in both herds were treated as soon as possible after detection. The nurse cows were changed from time to time and occasionally were alternated between the two groups. Four other frequently reinfected animals were likewise changed from one group to the other.

The normal milking procedure was to start with one milking unit on each of the infected cows in the hand-stripped group. After completing this group the same procedure was followed with the machine-stripped cows.

It was not possible to persuade the farmers to use only two machine units. With three and sometimes four units in use, certain cows, and not always the same ones, were not milked with an 'infected' unit. This, while unfortunate, is not considered to have influenced the results materially.

The farmers agreed to keep records of clinical symptoms at each milking and to send to the laboratory one or more S.P. Units (10) from clinical cases before treatment.

*Milk samples.* Individual quarter samples (first ½ oz.) from all milking cows were taken aseptically every 2–4 weeks. Weekly or twice weekly visits were made to obtain samples for confirmation of new infections, from dry cows and from animals before and after calving. When a cow was sampled a record was kept of teat sores and erosions.

Samples were plated on the surface of aesculin-sheep-blood-agar, using two wire loopfuls, i.e. about 0·05 ml. of fresh milk, and plates were examined after incubation for 48 hr. at 37° C. In most cases *Str. agalactiae* was identified by colony appearance; some cultures were confirmed by biochemical and serological methods and some sent to Dr A. W. Stableforth for typing. Other streptococci were kept for the usual confirmatory tests, and *Staphylococcus aureus* strains which exhibited no $\beta$ lysin on the primary plates were identified by coagulase tests. After refrigeration overnight the samples were examined for clots, sediment and other abnormalities.

*Treatment.* The initial treatment of quarters in herd Ba was by infusion of 100,000 units of penicillin in oil on three alternate days. After starting the investigation new *Streptococcus agalactiae* infections were treated within a few days of detection with an intramammary infusion of 300,000 units (three tubes) of penicillin or procaine penicillin. These new infections were assessed whenever possible on the basis of results of tests carried out on three milk samples. When a control cow developed clinical signs, attempts were made to suppress the symptoms without producing a bacteriological cure. This was

done by infusing about 5,000 units of penicillin into the quarter. Unfortunately, this often resulted in a bacteriological cure.

Quarters infected with other organisms were not treated unless they developed clinical symptoms.

*Infections.* Although an infection was normally assumed to be present when the pathogen was found in three or more samples, sometimes two samples, and in four instances one sample, were accepted. In the latter case the single samples were associated with clinical symptoms or with dry cows when large numbers of colonies were present.

In herd Ba *Staphylococcus aureus* was often present in small numbers in successive samples when the teat orifice was eroded. In such cases an intramammary infection was not assumed unless the number of colonies exceeded 500 per ml.

The sporadic occurrence of pathogens in the absence of clinical symptoms was regarded as due to temporary infections or contamination (false positives) from the skin of the teat.

*Cures.* Successful treatment was assumed if pathogens were not found: (1) in three or more samples, the first sample at least 1 week after treatment; (2) in two samples taken at least 1 and 4 weeks after treatment; and (3) if there was a change from haemolytic to non-haemolytic *Streptococcus agalactiae* or vice versa.

*Dry period.* New *Str. agalactiae* infections found at the first dry-period test have been regarded as lactation infections. This was done because an outbreak of foot-and-mouth disease in the district interrupted routine sampling for a short while, and a number of cows were not tested in the last 4–5 weeks of their lactation.

## RESULTS

Streptococcus agalactiae *infections.* The number of new infections which occurred in the hand-stripped and machine-stripped groups was twenty-five and twenty-four in herd Cr and seventy-seven and sixty-four in herd Ba (Table 1, row 17). A better estimate of the true effect of the treatments on the new infection rate is obtained from the sum of the number of weeks each cow was exposed to infection divided by the total number of new infections. This measure, the number of weeks of exposure per infection (Table 1, row 19), was 28·2 and 32·8 weeks for the hand-stripped and machine-stripped groups in herd Cr, and 9·5 and 9·6 weeks for the same groups in herd Ba. It is apparent from this data that the method of stripping had little effect on the new infection rates.

From Table 1, row 12, it will be seen that the average number of infections present at any one time in each experimental group was kept remarkably close within each herd, while the average level of infection in herd Ba was almost twice as great as in herd Cr. It is probable that this difference between herds is mainly due to the fact that the new infection rate per cow (Table 1, row 18) was four times greater in herd Ba even after allowance was made for the ages of the cows.

*Teat sores and erosions.* The proportion of teats with either small sores or erosions, near the teat orifice, was very significantly greater in herd Ba than in herd Cr, though the proportion in the hand- and machine-stripped group of each herd did not differ. On the other hand, in herd Cr the proportion of teats with small sores and in Ba the proportion of teats with frequent small sores on the body of the teats was significantly greater ($p < 0{\cdot}05$) in the hand-stripped group.

*Teat sores and new infections.* As might be expected, new infections with *Str. agalactiae* were significantly more frequent when the teat apex was abnormal than when it was

healthy. For instance, the percentage of samples revealing new infections and the number of samples taken from cows infected once or more were as follows: 2·7 and 1656 from healthy teats, 2·8 and 430 from teats with small sores not near the orifice, 7·9 and 241 from teats with erosions at the orifice and 8·2 and 219 from teats with small sores at or adjoining the orifice. Thus the presence of *small* sores on the body of the teat only did not

Table 1. *New* Streptococcus agalactiae *infections in machine- and hand-stripped cows in two herds and other relevant information*

| | | Herd Cr (strip-cup used) | | Herd Ba (no strip-cup) | |
|---|---|---|---|---|---|
| Row | Particulars | Hand-stripped | Machine-stripped | Hand-stripped | Machine-stripped |
| 1 | Duration of investigation, months | 12 | 12 | 13 | 13 |
| 2 | Total number of cows exposed to infection | 23 | 29 | 22 | 22 |
| 3 | Sum of number of weeks each cow was exposed to infection | 704 | 788 | 729 | 616 |
| 4 | Average of number of weeks each cow was exposed to infection | 31 | 27 | 33 | 28 |
| 5 | Number of cows exposed for more than 15 weeks | 20 | 22 | 19 | 18 |
| 6 | Number of cows exposed for less than 5 weeks | 0 | 5 | 2 | 3 |
| 7 | Number of cows which spent some time in both groups | 7 | | 7 | |
| 8 | First lactation animals, number infected/number examined | 2/9 | 2/10 | 0/4 | 2/6 |
| 9 | Average 'age' of cows (age in lactations assessed monthly) | 3·3 | 3·1 | 3·8 | 3·0 |
| 10 | Average number of cows exposed per month | 15·7 | 17·3 | 13·2 | 11·4 |
| 11 | Average number of quarters infected at the start of each month | 4·1 | 4·1 | 6·3 | 5·4 |
| 12 | Ditto, per every 10 cows exposed at the start of each month | 2·6 | 2·4 | 4·8 | 4·8 |
| 13 | Number of cows free from infection in lactation although exposed for 18–47 weeks | 8 | 11 | 4 | 4 |
| 14 | Number of cows with infections in lactation | 13 | 12 | 15 | 18 |
| 15 | Number of cows with new infections in lactation | 11 | 11 | 14 | 15 |
| 16 | Number of quarters with new infections in lactation | 19 | 19 | 41 | 40 |
| 17 | Number of new infections in lactation | 25 | 24 | 77 | 64 |
| 18 | New infections per cow* | 1·6 | 1·4 | 5·8 | 5·6 |
| 19 | Weeks of exposure per infection† | 28·2 | 32·8 | 9·5 | 9·6 |

* Total number of new infections divided by the average number of cows in milk per month.
† The sum of the number of weeks each cow was exposed to infection divided by the total number of new infections.

appear to influence intramammary infections in these herds; this is in agreement with Stuart & Lancaster(11). Very slight teat erosions were likewise unassociated with an abnormal new infection rate.

*Clinical incidence of* Streptococcus agalactiae *infections.* The proportion of clinical cases in machine- and hand-stripped groups is illustrated in Table 2. It will be seen that although there was little difference between the number of cows which became clinical in the two groups, the proportion of clinical quarters, clinical infections and the number of days on which clinical symptoms were recorded were greater in the machine-stripped group. This trend was particularly noticeable in herd Ba. Because of the relatively small number of cows in each group it is not surprising that the clinical effect could not be measured on a cow basis.

In the machine-stripped group in both herds, approximately the same proportion of infections caused clinical signs. In the hand-stripped groups, however, a much smaller

Table 2. *The proportion of clinical cases caused by* Streptococcus agalactiae *in lactating cows in the machine- and hand-stripped groups*

| | Herd Cr | | | | | |
|---|---|---|---|---|---|---|
| | Hand-stripped | | | Machine-stripped | | |
| | Total | Clinical | % | Total | Clinical | % |
| Infected cows | 13 | 7 | 54 | 12 | 8 | 67 |
| Infected quarters | 29 | 12 | 41 | 27 | 14 | 52 |
| Infections | 36 | 13 | 36 | 34 | 17 | 50 |
| Clinical cases reported by cowmen/total clinical cases found | | 9/13 | | | 16/17 | |
| Sum of clinical cases multiplied by the number of days each was clinical | | 25 | | | 51 | |
| | Herd Ba | | | | | |
| Infected cows | 15 | 10 | 67 | 18 | 16 | 89 |
| Infected quarters | 46 | 12 | 26 | 53 | 35 | 66 |
| Infections | 92 | 12 | 13 | 81 | 46 | 57 |
| Clinical cases reported by cowmen/total clinical cases found | | 0/12 | | | 8/46 | |
| Sum of clinical cases multiplied by the number of days each was clinical | | 23 | | | 111 | |

proportion was associated with a clinical condition in herd Ba than in herd Cr. Hand stripping seemed to be most prolonged in the former herd.

*Haemolytic and non-haemolytic strains of* Streptococcus agalactiae. Dr Stableforth reported that the haemolytic strains from both herds were type 3*a* and the non-haemolytic strain type 1*b*. Though we are not justified in assuming that all the other strains were of these types, it is of interest to compare the proportion of infections caused by haemolytic and non-haemolytic strains which gave rise to clinical signs (Table 3). From the table it

Table 3. *The proportion of clinical cases caused by haemolytic and non-haemolytic strains of* Streptococcus agalactiae

| | Herd Cr | | | | | | Herd Ba | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Hand-stripped | | | Machine-stripped | | | Hand-stripped | | | Machine-stripped | | |
| | Infections | Clinical | % | Infections | Clinical | % | Infections | Clinical | % | Infections | Clinical | % |
| Haemolytic strains | 36 | 12 | 33 | 34 | 17 | 50 | 54 | 6 | 11 | 60 | 36 | 60 |
| Non-haemolytic strains | — | — | — | — | — | — | 38 | 6 | 16 | 21 | 8 | 38 |
| Total | 36 | 12 | 33 | 34 | 17 | 50 | 92 | 12 | 13 | 81 | 46* | 57 |

* Two infections with mixed haemolytic and non-haemolytic strains.

will be seen that in herd Ba the higher clinical incidence in the machine-stripped group noted above was true for both haemolytic and non-haemolytic strains. There is no obvious explanation of the fact that the haemolytic infections showed a greater tendency to become clinical in the machine-stripped group, whilst the opposite was true for the hand-stripped group.

*Treatment.* The initial treatment of the twenty-one cows and fifty-eight quarters considered infected with *Str. agalactiae* gave 76 and 90% cures respectively. The results of the subsequent treatments (Table 4) indicate that treatment appeared to be less successful in herd Ba than in herd Cr. This may be a true difference between herds, or the result of

failing to distinguish between reinfections and relapses, which was materially more difficult in herd Ba where teat abrasions were more numerous.

Table 4. *Response of* Streptococcus agalactiae *infections to treatment with penicillin*

(With few exceptions treatment consisted of one dose of 300,000 units of penicillin infused through the teat orifice after evening milking.)

| | Herd Cr | | Herd Ba | | | |
|---|---|---|---|---|---|---|
| Lactating cows | Hand-stripped | Machine-stripped | Hand-stripped | Machine-stripped | Total | % |
| Number of infections treated in lactating cows | 21 | 24 | 74 | 69 | 188 | — |
| Cured by one dose of penicillin | 19 | 19 | 62 | 50 | 150 | 80 |
| Cured by two doses of penicillin | 2 | 4 | 6 | 13 | 25 | — |
| Cured by three doses of penicillin | 0 | 0 | 2 | 4 | 6 | — |
| Requiring more than three doses | 0 | 1 | 4 | 2 | 7 | 3·7 |
| Spontaneous recoveries | 3 | 1 | 3 | 2 | 9 | — |
| Percentage cured by one dose of penicillin | 85 | | 78 | | | |

*Infections caused by other organisms.* The total number of quarters infected in 12 months with *Staphylococcus aureus* and with streptococci of groups II and III (Minett), together with the number of quarters acquiring new infections during the lactation period, is shown in Table 5. The number of quarters with *Streptococcus agalactiae* is included in the table for comparative purposes.

Table 5. *A comparison between the number of quarters infected in* 12 *months with* Streptococcus agalactiae, *with other streptococci and with* Staphylococcus aureus

| | Herd Cr | | | | Herd Ba | | | |
|---|---|---|---|---|---|---|---|---|
| | Hand-stripped | | Machine-stripped | | Hand-stripped | | Machine-stripped | |
| Quarters of lactating and dry cows infected with: | No. | Clinical cases* | No. | Clinical cases | No. | Clinical cases | No. | Clinical cases |
| *Str. agalactiae* | 35 | 10 | 28 | 14 | 46 | 12 | 52 | 35 |
| Streptococci of groups II and III (Minett) | 21 | 2 | 20 | 0 | 17 | 2 | 9 | 2 |
| *Staph. aureus* | 9 | 3 | 8 | 2 | 26 | 6 | 22 | 6 |
| Quarters of lactating cows becoming infected with: | No. | %† | No. | % | No. | % | No. | % |
| *Str. agalactiae* | 19 | 31 | 19 | 27 | 41 | 78 | 40 | 88 |
| Streptococci of groups II and III (Minett) | 6 | 9·6 | 9 | 13 | 8 | 15 | 4 | 8·8 |
| *Staph. aureus* | 3 | 4·8 | 5 | 7·2 | 21 | 40 | 16 | 35 |
| Average no. of lactating quarters each month | 62·8 | | 69·2 | | 52·8 | | 45·6 | |

* Number of quarters with clinical signs in the lactation period, excluding the first 3 days after calving when the cows were not in the trial.

† Number of quarters infected, expressed as a percentage of the average number of lactating quarters each month.

There were differences in the percentage of quarters acquiring new infections in the hand- and machine-stripped groups, but these differences did not appear to be connected with the experimental treatment. As in the case of *Str. agalactiae* infections the incidence of *Staphylococcus* infections was much higher in herd Ba than in herd Cr.

There was no obvious difference in the proportion of clinical cases in the hand- and machine-stripped groups as there was, for example, in the case of *Streptococcus agalactiae* infections in herd Ba. In both the hand-stripped groups, the proportion of the *Staphylo-*

*coccus*-infected quarters with clinical signs was similar to that found for *Streptococcus agalactiae*, but a third of the former cases were mixed infections with *Str. agalactiae*.

Four cows in the hand-stripped groups subsequently developed severe dry-period infections from which *Corynebacterium pyogenes* was isolated, while one cow in the machine-stripped groups became infected with *Bacterium coli* (type I) which did not cause clinical symptoms.

*Strip-cup results.* The fore-milk in herd Cr only was examined for clots by the use of a strip-cup, and it is evident from the proportion of all clinical cases detected by the cowmen in the two herds (Table 2) that the strip-cup was of value. In both herds the cowmen reported most clinical cases in the machine-stripped group.

In herd Cr abnormalities were reported on eighty-two occasions from thirty-nine infections before treatment started, but minor clinical signs were sometimes found associated with apparently non-infected quarters (Table 6). For example, on forty-nine

Table 6. *Clinical signs found or reported in lactating quarters of herd Cr*

| Bacteria found | No. of infections or quarters with clinical signs | Small 'flakes' only | First reported as 'flakes' | First reported as hard or swollen | Hard or swollen at some time | Clots reported at some time |
|---|---|---|---|---|---|---|
| *Str. agalactiae* | 31 | 1 | 2 | 3 | 8 | 30 |
| *Str. uberis* | 5 | 1 | 1 | 0 | 0 | 4 |
| *Staph. aureus* | 4 | 1 | 2 | 0 | 1 | 2 |
| Total | 40 | 3 | 5 | 3 | 9 | 36 |
| Micrococci or/and diptheroids | 29 | 20 | 20 | 1 | 1 | 8 |
| None | 4 | 2 | 2 | 1 | 1 | 1 |
| Total | 33 | 22 | 22 | 2 | 2 | 9 |

occasions clinical signs were reported from thirty-three quarters with either no apparent infection or containing a few micrococci or non-haemolytic diptheroids; in only nine cases was the cow infected in another quarter. The frequency of these reports bore no relation to the stage of lactation or age of the cows, but the most definite signs (two quarters hard and swollen and three quarters with clots on four occasions) occurred in quarters of four newly calved cows; these cows were sampled frequently, but either no bacteria were found or only micrococci and diptheroids.

Bull, Murnane, Munch-Petersen & Maclean (12), McEwen & Cooper (13) and others, have discussed the nature and frequency of clots from quarters with minor infections or no evident infection. Transient infections with streptococci or staphylococci, however, might have been responsible for some of the cases.

*Clinical signs after treatment.* In herd Cr clots persisted for more than 1 day in the milk of 9% of forty-five infected quarters after the bacteria had been eliminated by treatment; in three quarters for 6–8 days and one quarter for 12 days.

## DISCUSSION

In the hand-stripped group, as both teat-cups and hands were contributing to the spread of *Streptococcus agalactiae*, and because of the greater incidence of teat sores, we should have expected the organism to be more widely distributed on teats and utensils. Archer (14) and others were also of the opinion that teat sores were most prevalent in hand-milked

herds. Although small sores on the teats were not found to be more often associated with new udder infections than were healthy teats, infected sores are known to be important though temporary reservoirs of *Str. agalactiae* and to add to the numbers of organisms in the environment.

It is thus somewhat surprising that we have not found in either herd a significant difference in the number of new infections in the two groups of cows. There are, however, a number of factors which need consideration. It is possible that stripping by hand increased the rate of spread of *Str. agalactiae*, but that this increase was balanced by an increased new infection rate in the machine-stripped group because of the greater clinical incidence (as clinical cases were more frequent, one would expect the number and virulence of the organisms to be increased (15)), or because the teat-cups were more heavily contaminated after use on the hand-stripped cows.

Moreover, even if the level of infection in hand-stripped herds is found to be greater than in machine-stripped herds this, in itself, does not necessarily mean that hand-stripping increases the new infection rate. The level of infection depends on the new infection rate and the recovery rate. From Table 2 it is evident that in the investigation, clinical cases occurred more frequently in the machine-stripped groups, and that if we had followed the normal practice of treating only clinical cases the level of infection in these groups would soon have been much lower than that in the hand-stripped groups. The higher incidence in the hand-stripped groups would in turn result in a greater new infection rate.

The planning, execution and interpretation of experiments on the effect of management practices on mastitis is inherently difficult. Apart from difficulties in controlling the degree of exposure to infection, equalizing the susceptibility of the animals in the control and treatment groups and pairing according to age, lactation stage and milk yield, there is always the difficulty of ensuring that the cowmen carry out their instructions, particularly in commercial herds. In this experiment we believe that exposure to infection was controlled fairly successfully (Table 1), and every effort was made to equalize the age and lactation stages of the two groups. The relative susceptibilities of the cows in each group was left mainly to chance, since there is no simple way in which this can be measured, and in order to supervise the cowmen visits were made to each herd on two or three occasions each week.

Whatever the cause(s) of the large difference in the infection rate between the two herds it is evident that it was more important than the experimental treatment within each herd. The only obvious differences between the two herds were in the teatcup liners, the personnel and the incidence of sores and abrasions near the teat orifice. It seems probable that infected teat sores were responsible for the high intramammary infection rate, for the relative incidence of clinical cases in the two herds did not differ greatly as might have been the case if the milking efficiency in the machine-stripped groups in the two herds had differed materially.

The observation that a greater proportion of the *Str. agalactiae* infections causing clinical signs occurred in the machine-stripped groups may be a reflexion of the efficiency of stripping. This seems quite probable in view of investigations of Ernst *et al.* (1), Schmidt-Hoensdorf & Schmidt (2) and Schalm & Mead (3), who found that clinical signs were least severe or/and less prolonged in udders which were efficiently stripped. That some observers (4, 5, 6, 7) have reported different results from these may possibly be because of variation in the completeness of stripping, the vacuum and the teat-cup liner.

In this investigation the percentage of clinical cases in the hand-stripped groups was lowest in herd Ba (Table 2) where hand-stripping appeared to be more prolonged than in herd Cr.

Another possible explanation of the results is that the milking machine damages the mucous membrane of the inner lining of the udder and teat sinus during machine stripping, and that this irritation predisposes the clinical state. It is reasonable to assume that while milk is flowing the inner lining of the teat is not subjected to the partial vacuum of the milking machine. Once, however, milk flow ceases or becomes intermittent, as in machine stripping, it is quite probable that the vacuum does have an effect within the udder.

The detection of clinical signs is important, as it is the only practical cowshed method of discovering infected quarters. Unlike the Elveden herd (16), where 90% of clinical cases found were detected without the aid of a strip-cup, only about 27% of *Str. agalactiae* cases in herd Cr were so detected and 14% in herd Ba. It is possible that with better observation more cases in herds Cr and Ba could have been detected without a strip-cup. Nevertheless, it is clear that the cowmen in herd Cr found their strip-cups most valuable. The cowmen in herd Ba should also have been using them.

The value of the strip-cup in detecting mastitis will not always be as great as in the herds used for this study. Unless a high proportion of the quarters yielding clots on the strip-cup are infected with the more pathogenic organisms (streptococci and staphylococci) its use is of doubtful value. Bull *et al* (12) came to the conclusion that the mere presence of clots could not be taken as a sign of infection, but this does not seem to be generally accepted. From the extensive observations of McEwen & Cooper (13) it appears that 12–13% of quarters yielding clots were not infected with the more pathogenic organisms. The comparable figure for herd Cr was 20% of the forty-five quarters yielding clots. The finding of a few small flakes only, in the milk of herd Cr, was not a reliable indication of infection with streptococci or staphylococci.

We are very grateful to Dr A. W. Stableforth for typing strains of streptococci, to Dr E. I. Garvie for identifying certain cultures, and to Miss H. R. Chapman and Mr P. A. Clough for their help.

REFERENCES

(1) Ernst, W., Schmidt-Hoensdorf, F. & Schmidt, W. (1931) *Arch. wiss. prakt. Tierheilk.* **63**, 401. Abstr. *Milchw. Forsch.* **12**, 151.
(2) Schmidt-Hoensdorf, F. & Schmidt, W. (1932). *Arch. wiss. prakt. Tierheilk.* **65**, 535.
(3) Schalm, O. W. & Mead, S. W. (1943). *J. Dairy Sci.* **26**, 823.
(4) Cullity, M. (1944). *J. Dep. Agric. W. Aust.* **21**, 228.
(5) Nichols, L. E. (1944). *Qd agric. J.* **59**, 357.
(6) Ward, A. H. (1944). *20th Ann. Rep. N.Z. Dairy Bd*, p. 49.
(7) Ward, A. H. & Castle, O. M. (1945). *21st Ann. Rep. N.Z. Dairy Bd*, p. 46.
(8) Edwards, S. J. (1951). Quoted by Wilson C. D. (1952). *Vet. Rec.* **64**, 525.
(9) Wilson, C. D. (1952). *Vet. Rec.* **64**, 525.
(10) Neave, F. K., Taylor, M. E. & Lawes, L. F. (1949). *Proc. Soc. appl. Bact.* (1), 34.
(11) Stuart, P. & Lancaster, J. E. (1949). *J. comp. Path.* **59**, 31.
(12) Bull, L. B., Murnane, D., Munch-Petersen, E. & Maclean, Jean D. (1940). *Bull. Coun. sci. industr. Res. Aust.* no. 134.
(13) McEwen, A. D. & Cooper, Margaret B. (1947). *Vet. Rec.* **59**, 655.
(14) Archer, R. T. (1915). *J. Dep. Agric. Vict.* **13**, 33.
(15) Holman, H. H., Pattison, I. H. & Taylor, J. I. (1950). *J. comp. Path.* **60**, 100.
(16) Neave, F. K., Phillips, Mary & Mattick, A. T. R. (1952). *J. Dairy Res.* **19**, 14.

(*MS. received for publication* 15 *July* 1953)

636.4:612.664

# 533. INTRAMAMMARY PRESSURE CHANGES IN THE LACTATING SOW

## I. THE EFFECT OF OXYTOCIN

By W. G. WHITTLESTONE

*Ruakura Animal Research Station, Department of Agriculture, New Zealand*

(With 9 Figures)

Changes in intramammary pressure in the cow have been measured by a number of workers(1, 2, 3). Such changes are of great interest in connexion with the problems involved in milking the cow. However, as a means of studying the mechanism of milk ejection they have certain limitations. The presence of an elastic cistern and ducts of substantial volume greatly modifies the changes in pressure which occur in the smaller ducts during the ejection process. This complicates the interpretation of such pressure changes in terms of the action of contractile elements within the secretory tissue. The sow offers a partial solution to this problem. Her mammary gland does not possess a large cistern and the collecting ducts are not large, so that the measurement of pressure changes within these ducts will probably follow the changes in the ductule-alveolar system (see Fig. 1).

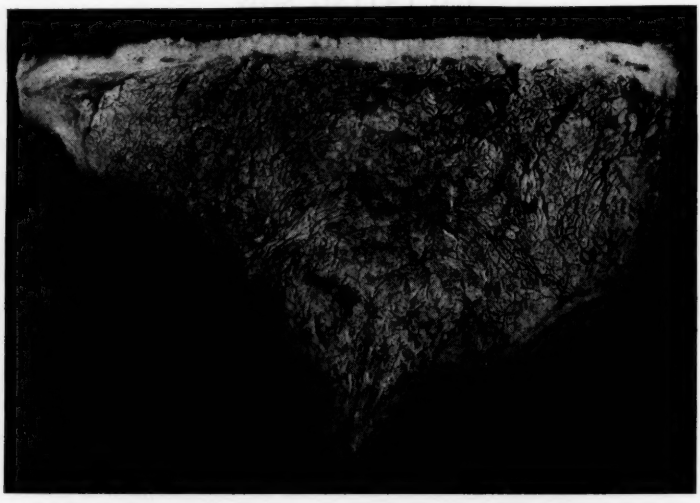
### METHOD OF MEASUREMENT

Because any marked displacement of liquid will introduce serious measurement errors, the apparatus should involve only small liquid movements, and if possible should be self-calibrating. Recording in ink on calibrated paper is a further desirable factor. The smoked drum, while simple, is most inconvenient when reliability and portability are essential. A mercury manometer was chosen as the pressure-sensitive element. One limb of this was made wide so that for practical purposes all of the movement occurred on one side. Within the narrow limb a small platinum resistance coil was fitted. This coil was wound on an alkathene former 50 mm. long and 3 mm. in diameter. The pitch of the coil was 0·5 mm. and the size of the wire 40 s.w.g., giving a resistance of 10 ohms approximately. The mercury in the manometer was connected to electrical earth via a small platinum electrode sealed into the manometer limb. The platinum resistance was connected to a high-voltage a.c. source through a high resistance, so that the small changes in its resistance due to the movement of the mercury caused a negligible change in current. Hence, the voltage output was proportional to the uncovered length of resistance and fell with rising pressure. The output after amplification (see appendix) was fed to a recording milliammeter. Because the voltage fell with rising pressure, the pressure zero on the paper was the full-scale reading. Should the pressure exceed the limits of the apparatus, the voltage falls to zero and no possibility of over-loading the recording instrument may arise.

Fig. 2 shows the construction of the manometer. It will be noted that a tail of wire projects below the coil into the mercury. This avoids the possibility of a high voltage being applied to the amplifier input should the pressure become negative. As a further

protection the platinum coil was shunted by a resistance within the power supply (see appendix). The system has a number of convenient features:

(1) By varying the amount of platinum wire above the mercury and by adjusting the



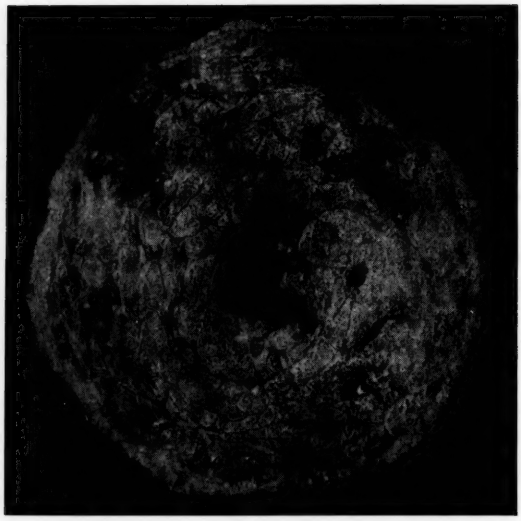


Fig. 1. Sections of sow mammary gland showing duct system.

gain control of the amplifier, the range from zero to full scale may be altered from 0 to 50 mm. of mercury down to 0 to 10 mm. or lower.

(2) The system is self-calibrating in that it measures the pressure as a head of mercury.

(3) By using different lengths of coil the maximum range may be varied quite substantially.

The apparatus was connected to the sow via a rubber tube and a teat siphon made from a stainless steel hypodermic needle. The needle was blocked at one end with 'easy-flow' low-melting brazing alloy and shaped to a smooth taper with a rounded end.

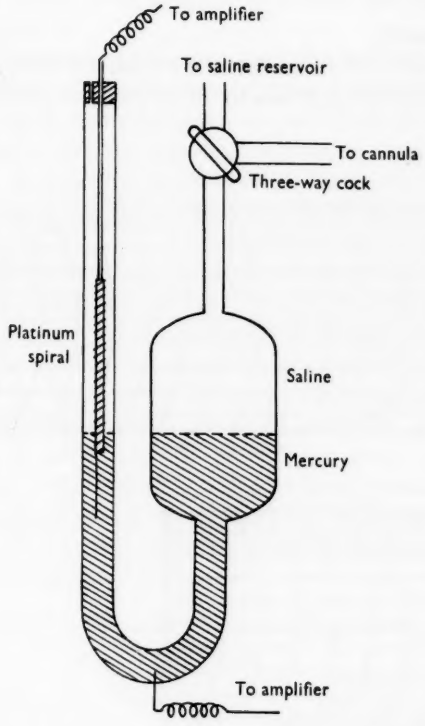



Fig. 2. Manometer used in pressure measurements.

Two small slots were then formed in the sides about 7 mm. apart. The most useful size proved to be 1·2 mm. diameter and 35 mm. long. A convenient means of holding the siphon in position was found to be a length of elastic bandage with a small slit cut in it to accommodate the lower end of the siphon. The system between the siphon and the manometer was filled with sterile saline, and normally there was a head of a few inches of saline between the gland and the manometer.

### THE EFFECT OF OXYTOCIN ON THE SOW'S INTRAMAMMARY PRESSURE

In the following experiments oxytocin was injected into an ear vein of a lactating sow via a siliconed needle, which was left in position between injections. One front gland was connected to the pressure recorder, and an adjacent gland hand-milked to measure the delay between injection and ejection and the duration of ejection(4).

*Experiment* 1

Fig. 3 sets out a series of pressure responses for the following treatments:

1. 0·5 i.u. oxytocin.
2. 0·5 i.u. oxytocin.
3. 0·5 i.u. whole pituitrin.
4. 0·5 i.u. oxytocin.
5. 0·5 i.u. whole pituitrin.
6. 0·4 i.u. oxytocin followed 10 sec. later by 0·4 i.u. oxytocin.
7. 0·4 i.u. oxytocin followed in 20 sec. by 0·4 i.u. oxytocin (the syringe leaked slightly).
8. Repeat of no. 7.
9. 0·5 i.u. of oxytocin.

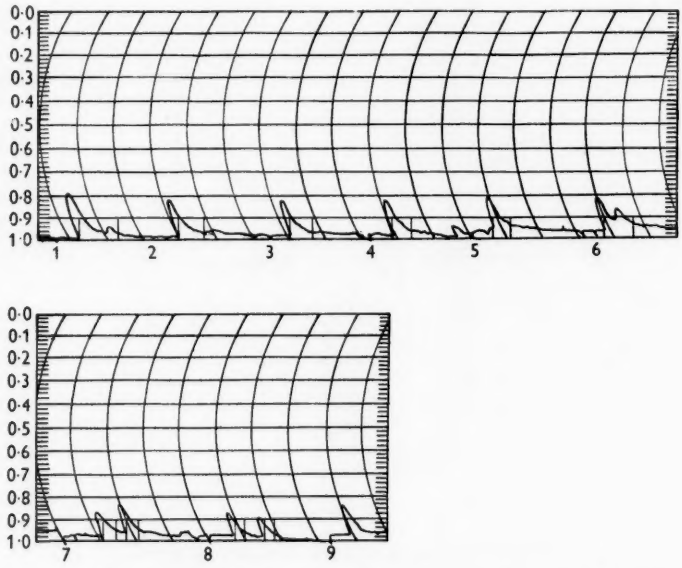



Fig. 3. Recordings of sow intramammary pressure changes following injections of oxytocin and whole pituitrin. The responses correspond with the following injections: 1, 0·5 i.u. oxytocin; 2, 0·5 i.u. oxytocin; 3, 0·5 i.u. whole pituitrin; 4, 0·5 i.u. oxytocin; 5, 0·5 i.u. whole pituitrin; 6, 0·4 i.u. oxytocin followed by the same dose in 10 sec.; 7, 0·4 i.u. oxytocin followed by the same dose in 20 sec. (some leakage occurred); 8, repeat of no. 7; 9, 0·5 i.u. oxytocin. Calibration: time intervals 30 sec., five small pressure intervals = 17 mm. Hg.

The downward 'pips' on the graph indicate the point at which the injection was made. The injections were made at approximately 5 min. intervals. The paper drive of the recorder ran for a few seconds before injection until the pressure had returned to normal. Movements of the sow while the recorder was not running account for the occasional steps in the graph.

The first three graphs show an unmistakable secondary rise in pressure 25·0, 22·5 and 22·5 sec. respectively after the initial pressure rise. The delay between injection and ejection in these sows was 17·4, 19·6, and 19·3 sec. (Table 1). The secondary rise was probably due to a return of a reduced peak of concentration of the hormone in the blood.

When this phenomenon occurred there was a definite 'double release' of milk observable when hand-milking. These observations seem to demonstrate a total circulation time compared with the time taken for blood to flow from the ear vein to the mammary gland (5).

Graph 6 shows the effect of two injections spaced by an interval of 10 sec. The graph shows the pressure rises caused by these injections to be spaced by 12·5 sec. When the delay between injections was 20 sec. as in graphs 7 and 8, the responses were spaced by 22·5 and 25·0 sec. respectively.

All responses were characterized by a very steep pressure rise and a much slower fall. A check on the characteristics of the recording apparatus showed that for both rising and falling pressures the possible recording speed was much greater than any changes so far recorded, so that the curves were not influenced significantly by the characteristics of the instrument except for the small liquid displacement involved. The rounded tops of the pressure waves are probably due to the resistance offered to the movement of liquid by the duct system.

In Table 1 the periods between injection and ejection and the duration of ejection, as determined from hand-milking, are set out. The duration of the ejection periods are shown in Fig. 3 as a series of pairs of light vertical lines. The end of hand-milking coincided with the point where the pressure curves started to flatten. This would suggest that the slow 'tail' on the pressure curve is due to the fact that when the ducts have closed (6) and the alveoli completely relaxed there is a slow return to equilibrium between the liquid in the recording equipment and that in the larger ducts. It is interesting to note that the amplitude of the pressure waves remained relatively constant for the five simple injections, while the duration of ejection decreased (4). It is also significant that whole pituitrin had the same effect as oxytocin when given in the same number of units. This is being investigated more thoroughly.

Table 1. *Hand-milking data—Experiment* 1

| Injection no. | Period between injection and ejection (sec.) | Duration of ejection (sec.) |
|---|---|---|
| 1 | 17·4 | 32·2 |
| 2 | 19·6 | 20·2 |
| 3 | 19·3 | 17·5 |
| 4 | 22·6 | 16·1 |
| 5 | 21·0 | 14·1 |
| 6 | 21·4 | 24·2 |
| 7 | 21·8 | 10·2 and 10·8 |
| 8 | 22·0 | 9·0 and 8·5 |
| 9 | 22·5 | 7·3* |

* This abnormal result was due to the removal of more milk from the gland than was secreted during the experiment. Normally no more than five milking tests were done at one session, and the amount of milk actually removed kept as small as possible, when the sow was used for assay purposes.

*Experiment* 2

In this case the recorder was operated at a higher sensitivity than in Exp. 1 and a positive head was maintained on the manometer. The same sow was used. The pressure graph is given in Fig. 4. The following are the data describing the injections:

1. 0·5 i.u. oxytocin.
2. 0·5 i.u. oxytocin.
3. 0·5 i.u. whole pituitrin.

4. 0·5 i.u. oxytocin (syringe leaked).
5. 0·5 i.u. oxytocin (syringe leaked).
6. 0·5 i.u. whole pituitrin.
7. 0·4 i.u. oxytocin (syringe broke).
8. 0·4 i.u. oxytocin followed by same dose in 5 sec.
9. 0·8 i.u. oxytocin given in 0·2 i.u. lots at intervals of 1 sec.
10. 0·8 i.u. oxytocin given continuously over a period of 8 sec.

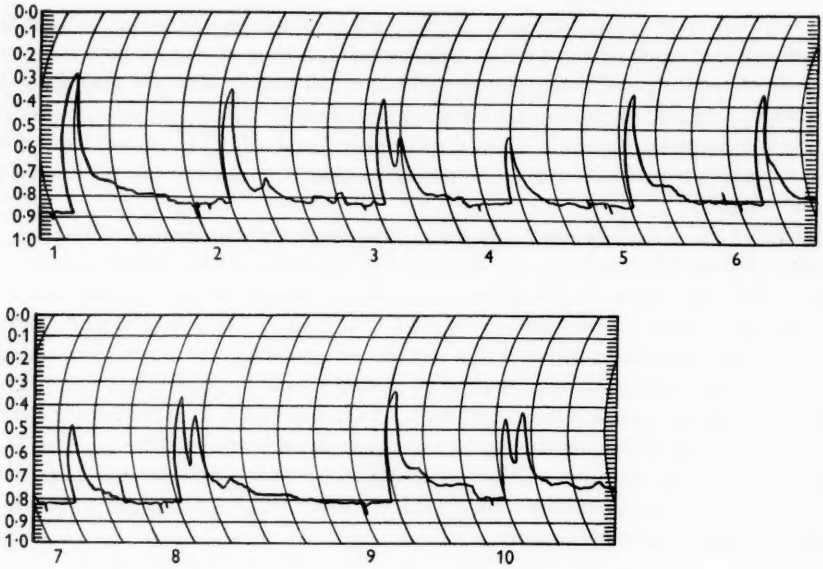



Fig. 4. Recordings of sow intramammary pressure changes following injections of oxytocin and whole pituitrin. Treatments: 1, 0·5 i.u. oxytocin; 2, 0·5 i.u. oxytocin; 3, 0·5 i.u. whole pituitrin; 4, 0·5 i.u. oxytocin (syringe leaked); 5, 0·5 i.u. oxytocin (syringe leaked); 6, 0·5 i.u. whole pituitrin; 7, 0·4 i.u. oxytocin (syringe broke); 8, 0·4 i.u. oxytocin followed by the same dose in 5 sec.; 9, 0·8 i.u. oxytocin given in 0·2 i.u. lots at intervals of 1 sec.; 10, 0·8 i.u. oxytocin given continuously over a period of 8 sec. Calibration: Time intervals 30 sec., five small pressure intervals = 5 mm. Hg.

Table 2 sets out the hand-milking data.

Table 2

| Injection no. | Period between injection and ejection (sec.) | Duration of ejection (sec.) |
|---|---|---|
| 1 | — | 29·3 |
| 2 | 20·2 | 21·9 |
| 3 | 19·5 | 32·1 |
| 4 | — | — |
| 5 | 21·7 | 18·5 |
| 6 | 20·4 | 17·9 |
| 7 | — | — |
| 8 | 19·2 | 27·7 |
| 9 | 26·0 | 20·2 |

The first pressure graph shows a normal pressure rise, but the falling portion has an abnormal foot. This was possibly due to a second ejection. Such a second response occurred in the second graph about 23 sec. after the first rise. The secondary rise was

somewhat protracted and suggests a substantial spread in the volume of blood occupied by the hormone. Graph 3 is somewhat unexpected in form. A possible explanation is that the injection was partly obstructed and as a result the hormone passed slowly into the blood stream. Graph 4 is of little value because of leakage of the material. Graph 5, like 1, shows a distinct foot. In the case of 6 there is a slight foot. Again it seems that whole pituitrin, despite its pressor content, exerted the same action as purified oxytocin. The seventh graph may be neglected, because of a mishap causing loss of liquid. In the case of the double curve, 8, the period between two equal injections was 5 sec. The peaks of the pressure maxima were 18 sec. apart, and the beginnings of the rise in pressure 16 sec. apart. This suggests some sort of latency in the reaction of the mammary myo-epithelium to oxytocin. In the case of 9, when the oxytocin was injected in 0·2 i.u. steps at 1 sec. intervals for 4 sec., the response curve was more like the reaction to a simple injection shown in graphs 1 and 5. When 0·8 i.u. of oxytocin was injected continuously over a period of 8 sec. as in graph 10, there was again a biphasic response with the peaks 14 sec. apart. Because it was thought that the earlier double graph (no. 3) may have been due to an injection artefact the tenth injection was carried out with exceptional care. The hormone solution appeared to flow gradually into the vein, and the peculiar response is thought to be really due to the reactions of the mammary gland. The shorter period between the peaks and the similarity in amplitude seem to rule out the explanation offered for the small secondary responses such as that shown in graph 2.

*Experiment* 3

The effect of varying the amount of oxytocin injected on the pressure record is given in Fig. 5, while Table 3 sets out the hand-milking data. The same sow was used in this experiment as in the foregoing.

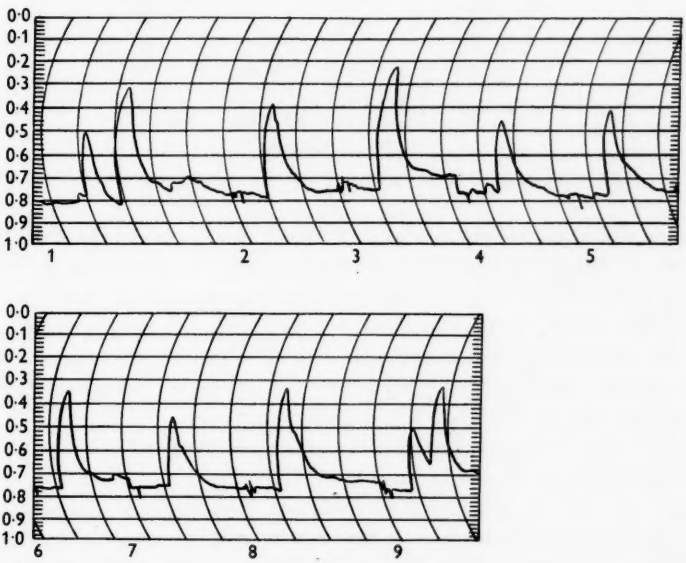



Fig. 5. The effect of varying the amount of oxytocin injected on the intramammary pressure of the sow (see Table 3). Calibration: same as Fig. 4.

Table 3

| Injection no. | Dose (i.u.) | Interval between injection and ejection (sec.) | Duration of ejection (sec.) | Pressure rise (mm. Hg) |
|---|---|---|---|---|
| 1 | 0·5 | 24·3 | — | — |
| 2 | 0·4 | 20·8 | 24·5 | 19·5 |
| 3 | 0·6 | 25·3 | 25·0 | 26·0 |
| 4 | 0·2 | 27·9 | 14·9 | 15·0 |
| 5 | 0·3 | 25·3 | 17·3 | 18·0 |
| 6 | 0·5 | 22·8 | 16·4 | 20·5 |
| 7 | 0·1 | 28·9 | 15·8 | 14·5 |

Owing to a sudden movement of the sow, the first injection was given in two steps. The graph for this must therefore be neglected. All other injections were normal. The duration of ejection is compared with the pressure rise in response to different doses of oxytocin in Fig. 6. The change in pressure seems a little more consistent than the duration of ejection, but the data so far are not adequate for a comparison of the two as a basis for an assay method.

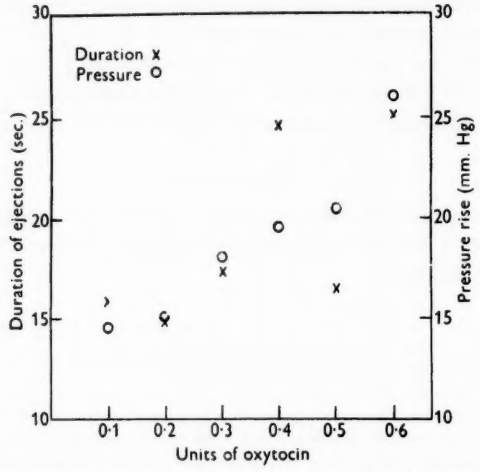



Fig. 6. A comparison between duration of ejection and pressure rise following different doses of oxytocin.

Pressure graphs 8 and 9 show the reaction to 0·8 i.u. of oxytocin injected over periods of 8 and 16 sec. Graph 8 appears to be an abnormally small response to such a dose. The next graph, however, shows a marked double response with the pressure starting to rise at two points 20 sec. apart. This effect may have been due to circulation, though the fact that the second response was larger than the first and the injection continued over such a long period, suggests strongly that there was a period of relaxation despite a level of hormone high enough to initiate a very definite response.

A second sow was used for the experiment illustrated by Fig. 7. The doses of pituitrin given and other data are set out in Table 4.

This sow was more sensitive to whole pituitrin than the one previously used. The reaction to 0·5 unit sent the pressure up beyond the limits of the recorder. Responses to various doses are set out in graphs 2–6. Fig. 8 shows again that the pressure response may be more regularly related to the hormone concentration than the duration of milk-flow.

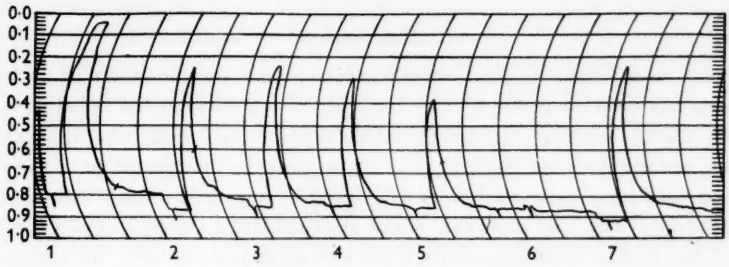



Fig. 7. The effect of varying doses of pituitrin on the intramammary pressure of the sow (see Table 4). Calibration: same as Fig. 4.

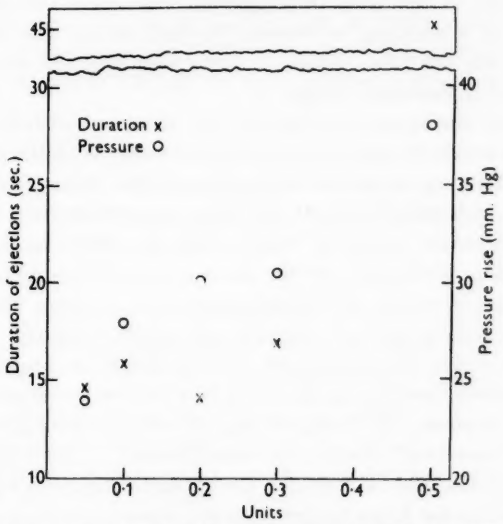



Fig. 8. A comparison between duration of ejection and pressure rise following different doses of pituitrin.

Table 4

| No. of injections | Dose (i.u.) | Injection-ejection interval (sec.) | Duration (sec.) | Pressure rise (mm. Hg) |
|---|---|---|---|---|
| 1 | 0·5 | 14·1 | 45·1 | 38·0 |
| 2 | 0·2 | 17·2 | 14·1 | 31·0 |
| 3 | 0·3 | 15·7 | 17·0 | 30·5 |
| 4 | 0·1 | 16·7 | 15·9 | 28·0 |
| 5 | 0·05 | 17·1 | 14·6 | 24·0 |
| 6 | 0·025 | — | — | — |

The point at which the hand-milking was judged to be complete is marked on the pressure curves as a small line at an angle to the time axis. In this case there was considerable variation in the relation between the shut-off point and the pressure-change curve. It appears that a dose of 0·025 unit was below the threshold required to initiate a response. This is another point on which more work is being carried out. The final graph, no. 7, is the result of injecting 0·4 unit in 16 sec. The pressure wave was simple and showed no abnormality other than a slower return to pre-injection pressure than the remainder of the graphs.

## DISCUSSION

The absence in the past of good experimental animals for the study of milk-ejection phenomena has been overcome by the discovery of the value of the breeding sow. Her mammary glands are large enough to enable fairly robust measuring apparatus to be used, they are simpler than those of the cow, so that the interpretation of the results is easier, and she is temperamentally very well suited for such work. Admittedly the question of how accurately one may generalize about mammals from the reactions of the sow may be raised, but this problem arises with respect to all animals but the cow and goat, if we approach the problem from a practical point of view.

The yields from many hundreds of routine machine milkings compared with normal sucklings carried out at the Ruakura Piggery(7) show that the amount of oxytocin released normally at a suckling is between 0·5 and 1·0 i.u. The duration of natural suckling times also fits this view. Hence, it would seem that the observations described come within normal physiological range.

A few preliminary observations of the sow just prior to parturition suggest strongly that the ejection hormone is in fact the oxytocic hormone. With the onset of labour milk ejection occurs. Following an obvious contraction of the uterus, it is possible to detect the ejection of milk by hand-milking. In fact, this is an excellent way to obtain colostrum samples. Hence, it seems logical to believe that the effects in the mammary gland following the injection of oxytocin are the same as those following natural ejection. So far this latter phenomenon has not been recorded on the pressure-measuring equipment, though this will be done as soon as a suitable sow presents herself.

One of the most evident characteristics of the milk-ejection process in the sow is the suddenness with which the pressure rises and falls. Attempts to prolong this rise may result in a biphasic response, which suggests that after the initial triggering there is a phase of relaxation and insensitivity to the hormone followed by a further response. If the mechanism of ejection in the cow is similar, the apparent duration of the normal response in this animal would be due to a sequence of pulses of pressure. Normal ejection duration in the cow varies from 2 to 10 min.(8). Unless the bovine myoepithelium is different in its reaction to oxytocin such a duration would involve many contraction cycles. Another possibility is that there is only one or a small number of actual ejections, and that the apparent duration of flow from the alveoli is actually flow from distended ductules from which the milk moves in mainly one direction.

A surprising feature of the reaction to oxytocin is the fact that an injection appears to pass as a concentration gradient twice through the circulatory system without becoming totally diffused. It seems that the concentration required to trigger off the ejection process is very much greater than that which would result from a complete mixing. Hence, it would be very difficult to detect the presence of the hormone in a blood sample unless it were taken just as the peak of concentration passed. There is evidence(9, 10) that when milk ejection occurs the whole posterior-lobe hormone group is released. This unitary theory involves the view that the release of anti-diuretic activity also involves the oxytocic fraction. The present observations would be compatible with this view because while the milk ejection and presumably the uterus-contracting functions involve a peak of concentration, the anti-diuretic action occurs at a much lower level of concentration. The control of diuresis could thus continue without producing any effect on the mammary gland.

Work continues on the factors controlling the ejection-pressure effects in the sow mammary gland. The preliminary work described suggests that much useful information may be gleaned by the use of this animal. It is of course logical to apply similar methods to the cow, but until the picture of the mechanism in the simpler gland system of the sow is clear, it is difficult to interpret the quite different pressure changes which occur in the cow.


## SUMMARY

Using an electronic pressure recording system, pressure changes in the sow's mammary gland in response to injections of oxytocin and whole pituitrin have been recorded. The results suggest that an injection of oxytocin within physiological range may pass round the circulatory system twice, resulting in a small secondary pulse of pressure. Slow injections over periods of the order of 8–16 sec. are shown to produce a double rise in pressure, the two phases of which are of the same order of magnitude, suggesting a contraction followed by a period of relaxation and a further contraction.


Thanks are due to Mr D. M. Smith for the use of the sows, Mr E. D. O'Reilly for skilful technical assistance and Mr R. Parkinson for time measurements during the recording of the pressure graphs. The photograph of the gland sections was taken by Mr J. Gibson, and the drawings done by Mr B. H. Millar.

## REFERENCES


(1) TGETGEL, (1926). *Schweiz. Arch. Tierheilk.* **68** (7), 335.
(2) TURNER, C. W. (1935). *Mo. agric. Exp. Sta. Bull.* no. 346.
(3) PHILLIPS, D. S. M. (1950). *Proc. N.Z. Soc. Anim. Prod.* p. 81.
(4) WHITTLESTONE, W. G. (1952). *J. Endocrin.* **8**, 89.
(5) WHITTLESTONE, W. G. (1953). *J. Dairy Res.* **20**, 13.
(6) WHITTLESTONE, W. G. (1950). *Nature, Lond.*, **166**, 994.
(7) SMITH, D. M. (1953). *Private communication.*
(8) PHILLIPS, D. S. M. (1953). *Private communication.*
(9) PEETERS, G. & COUSSENS, R. (1950). *Arch. int. Pharmacodyn.* **84**, 209.
(10) CROSS, B. A. (1950). *Nature, Lond.*, **166**, 612.


## APPENDIX

### *Amplifier used with the pressure recording system*

The amplifier used for the recordings described in this paper was one designed to drive a large 0–20 mA. instrument. Because of the size and unsuitability of this instrument for portable applications, a smaller 0–1 mA. unit was used with the original amplifier. It was thought useful to describe the apparatus because of its convenience and reliability. Fig. 9 gives the circuit. The first stage is a pentode (connected as a triode for this application) followed by another pentode. This drives a beam power tube capable of quite a substantial output for driving recording or controlling equipment. The a.c. output is rectified by a selenium rectifier to give the necessary d.c. signal. Because of the non-linearity of the rectifier used and variations in amplification with changes in heater voltage due to line fluctuations, approximately 19 d.b. of negative feed-back is applied from a resistor in the return circuit of the rectifier to the cathode circuit of the second voltage amplifier.

This degree of feed-back was made possible by by-passing high frequencies between the plate and cathode of the second amplifier, thus avoiding positive feed-back and resultant instability due to phase shift at high frequencies in the output transformer and rectifier.

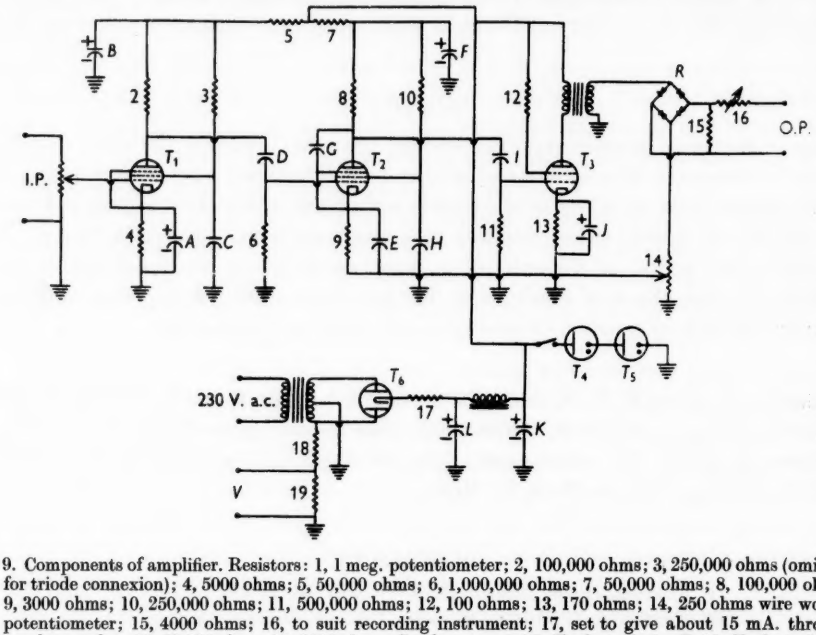



Fig. 9. Components of amplifier. Resistors: 1, 1 meg. potentiometer; 2, 100,000 ohms; 3, 250,000 ohms (omitted for triode connexion); 4, 5000 ohms; 5, 50,000 ohms; 6, 1,000,000 ohms; 7, 50,000 ohms; 8, 100,000 ohms; 9, 3000 ohms; 10, 250,000 ohms; 11, 500,000 ohms; 12, 100 ohms; 13, 170 ohms; 14, 250 ohms wire wound potentiometer; 15, 4000 ohms; 16, to suit recording instrument; 17, set to give about 15 mA. through regulator tubes; 18, 20,000 ohms; 19, 1000 ohms. Condensers: *A*, 25 $\mu$F. electrolytic; *B*, 16 $\mu$F. electrolytic; *C*, 0·5 $\mu$F. paper (omitted for triode connexion); *D*, 0·05 $\mu$F. paper; *E*, 25 $\mu$F. electrolytic; *F*, 16 $\mu$F. electrolytic; *G*, 0·004 $\mu$F. mica; *H*, 0·5 $\mu$F. paper; *I*, 0·05 $\mu$F. paper; *J*, 25 $\mu$F. electrolytic; *K*, 16 $\mu$F. electrolytic; *L*, 16 $\mu$F. electrolytic; *R*, selenium full-wave rectifier; $T_1$, 6SJ7 (used as a triode in the present application); $T_2$, EF36; $T_3$, 6L6G; $T_4$, VR 150/30; $T_5$, VR 105/30; $T_6$, 5Z3.

The plate supply is regulated with two gas tubes. Because of the heavy current drawn by the power tube, a switch is placed in the regulator tube circuit so that these tubes do not draw current until the power tube is warmed up after switching the apparatus on.

The combination of a high level of negative feed-back and a regulated high-tension supply has made the amplifier more than accurate enough for the purposes to which it has been applied.

(*MS. received for publication* 23 *April* 1953)

637.128:576.3

# 534. THE CELLS IN BOVINE MILK

By P. S. BLACKBURN* and IAN MACADAM†
*The Department of Pathology, The Western Infirmary, Glasgow*

(With 4 Figures)

This work was carried out to determine the types of cell found in normal and abnormal milk from cows. In the early part of the present century, the cell count was used by many public-health workers as an indication of the hygienic quality of milk. There was doubt, however, as to the exact significance of the various types of cell present, particularly whether they constituted pus or not. Thus Pennington & Roberts (1) assumed that all cells found in milk were leucocytes or pus cells, and therefore concluded that 75% of cows towards the end of lactation showed pus in the milk. On the other hand, Revis (2) thought that even normal milk could contain many leucocytes and so they were of no pathological significance. Hewlett, Villar & Revis (3) believed that the majority of the cells in milk differed considerably from leucocytes, and although many were multinucleate, they were not polymorphs and there was little phagocytic action. In the following year the same workers (4) examined 100 smears from the milk of six cows and observed no cells resembling polymorphs and so concluded that the main cells in milk were epithelial. They found high cell counts to be due mainly to the presence of large numbers of multinucleate cells. Savage (5) found leucocytes in milk at all stages of lactation. He recognized polymorphs, lymphocytes and large leucocytes and noted up to 70% of the last-named in the milk of cows at the end of lactation. Scannell (6) stated that leucocytes present in small numbers in milk did not constitute pus, but only if they were abundant or associated with streptococci. Breed (7) thought that the cells in milk were leucocytes and epithelial cells. Varrier-Jones (8) thought that dead polymorphs in milk constituted pus, but this was not so in the case of active polymorphs. He gave a detailed description of nine types of cell which he found in bovine milk. Bourgeois (9) stated that in normal milk the ratio of multinucleate cells to mononucleate was approximately 1:1. Holm (10) stated that normal milk contained only large and small lymphocytes, and that polymorphs and monocytes were present only in milk from cows suffering from mastitis. Zlotnik (11) considered that the multinucleate cells found in normal milk were degenerated epithelial cells or 'pseudopolymorphs'.

## MATERIALS AND METHODS

Total and differential cell counts were carried out on more than 5000 samples of fore-milk from normal and mastitis cows in various stages of lactation. The milk was examined by the direct-smear method of Prescott & Breed (12). The milk, after being examined by culture methods, was immediately formalized, one drop of 40% formaldehyde being added for each 10 ml. of milk.‡ This treatment not only preserved the milk and fixed the

* Now at the Hannah Dairy Research Institute, Kirkhill, Ayr.
† Present address: c/o Veterinary Department, P. O. Vom, Northern Nigeria.
‡ Hewlett *et al.* (4) also formalized the milk in this way on the assumption that this treatment increased the number of cells in the centrifuge deposit.

cells but also lessened the risk of the smears leaving the slide during staining. To prepare smears, 0·01 ml. of milk was transferred by means of a standard platinum loop to a microscope slide and smeared over an area of 1 cm.². Four separate smears, one from each quarter of the udder, were made on the same slide. These smears were dried on a level hot-plate and defatted by immersion for 2 min. in tetrachlorethane, then drained and dried. The dried smears were immersed in the Sudan Black B solution used by Sheehan & Storey (13) for staining the cytoplasmic granules in granular leucocytes.* After 20 min. in this solution the smears were treated with 0·1 % acid alcohol for 30 sec. to remove the stain from any fat globules that were still present. The preparation was washed in water for 2 min., drained and dried and counterstained by Leishman's method.

## RESULTS

The cells found in the milk smears stained by Sudan Black, Leishman method, may be classified as follows:

(1) *Granular leucocytes.* These cells contain cytoplasmic granules stained dark brown. This group includes the polymorph, the eosinophil and perhaps the basophil, but it was not possible to identify the three cells by the method used. These cells (see Fig. 1) may be

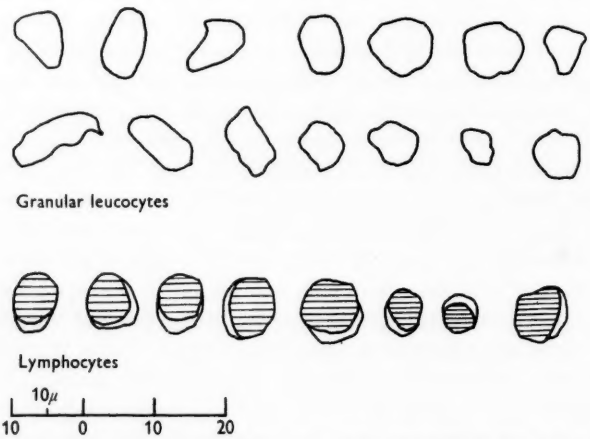



Fig. 1. Camera-lucida drawings of granular leucocytes and lymphocytes, as seen in a Prescott & Breed smear of milk treated with formaldehyde.

rounded, from 5 to 6 $\mu$ in diameter, oval $5 \times 3\mu$ to $9 \times 7\mu$ or elongated $9 \times 4\mu$ to $13 \times 3\mu$. The elongated type are found only in smears prepared from milk which has been formalized soon after it is drawn from the cow. Fig. 2 shows the comparative sizes of granular leucocytes as they appear in a blood film, in a thin smear prepared from unformalized milk and in a Prescott & Breed smear prepared from unformalized milk. It will be seen that the cells appear much larger in the thinner films, and that the addition

* (1) Stock solution of Sudan Black: Sudan Black B, 0·3 g.; absolute alcohol, 100 ml. Leave the solution for a few days to allow the dye to dissolve.

(2) Stock buffer solution: phenol, 16 g.; absolute alcohol, 30 ml.; sodium hydrogen phosphate ($Na_2HPO_4$), 0·3 g.; distilled water, 100 ml. Make separate solutions of the phenol in the absolute alcohol and of the sodium hydrogen phosphate in the distilled water and then mix the two solutions. For staining purposes 40 ml. of the buffer solution is mixed with 60 ml. of the stock solution of Sudan Black. The stain can be used at once.

of formalin to the milk (Fig. 1) causes a marked reduction in size. For this reason the cells in blood films are much larger than those in smears of formalized milk.

(2) *Non-granular cells.* These cells contain no dark brown granules in the cytoplasm. This group includes:

(*a*) Lymphocytes measuring 5–9 $\mu$ in diameter (see Fig. 1).

(*b*) Non-vacuolated epithelial cells 7–15 $\mu$ in diameter and with large nuclei (see Fig. 3).

(*c*) Vacuolated epithelial cells measuring 9–24 $\mu$ (see Fig. 4).

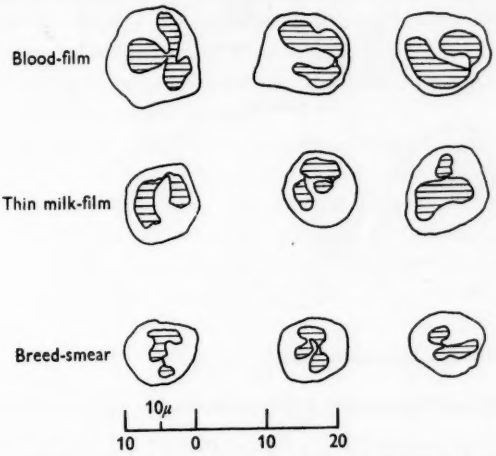



Fig. 2. Camera-lucida drawings of granular leucocytes in blood films, thin milk smears and Prescott & Breed smears. The milk smears were prepared from unformalized milk.

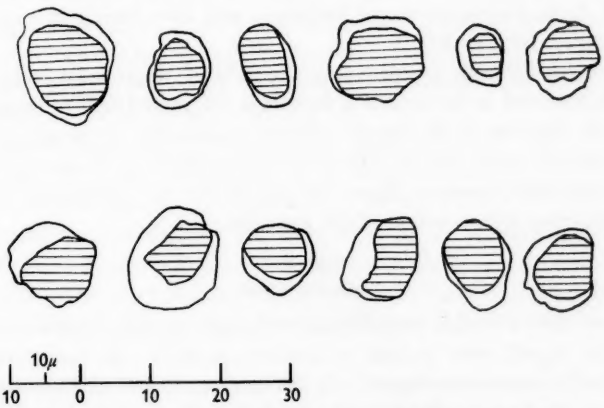



Fig. 3. Camera-lucida drawings of non-vacuolated epithelial cells.

It is possible that other cells (monocytes, macrophages and plasma cells) occur in milk but they have not been recognized in the present investigation.

Granular leucocytes, lymphocytes and vacuolated and non-vacuolated epithelial cells were found both in milk from mastitis udders, and in milk from udders in which no inflammatory lesions were found on histological examination. Granular leucocytes were

seldom absent from the smears. Even when only a few cells were found in a smear, one at least of these was almost invariably a granular cell. In the early part of this investigation before formaldehyde was used to preserve the milk multinucleate degenerated epithelial cells were occasionally observed, but never in such numbers as described by Zlotnik (11).

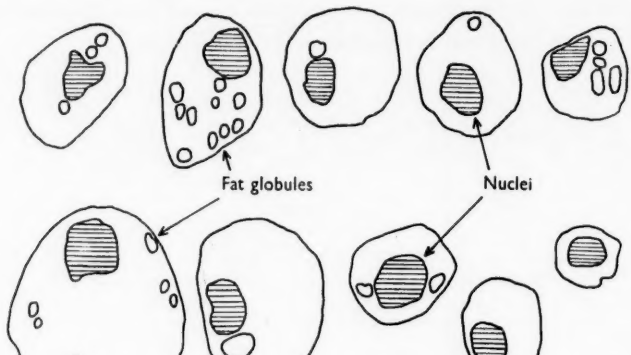



Fig. 4. Camera-lucida drawings of large epithelial cells with vacuolated cytoplasm.

The non-vacuolated epithelial cell is the cell cast off from the acini and is found in practically all milk smears. It becomes more abundant in advanced lactation, being then cast off in the process of involution. In sections of udder tissue large numbers are seen in the acini and ducts during advanced lactation, and also during the rapid process of *post-inflammatory involution* (14).

Vacuolated epithelial cells are probably the cells of 'suppressed lactation', in other words the cells observed in the acini if a ductule is obstructed or if milking of the animal is suspended (15). Spencer & McNutt (16) refer to vacuolation of the acinar epithelium in acute lobular mastitis, and consider it to be due to the blocking of a ductule by coagulated exudate. In some cases, however, these cells may be the vacuolated epithelial cells found in subacute catarrhal inflammation of the ducts (14).

## DISCUSSION

Varrier-Jones (8) used a double centrifuging technique to concentrate the cells in milk, and films of the deposit were stained by Jenner's method. He described nine types of cells: (1) *the finely granular eosinophil cell*, (2) *the coarsely granular eosinophil cell*, (3) *the basophil cell*, (4) *the large neutrophil cell*, (5) *the lymphocyte*, (6) *the red blood corpuscle*, (7) *the large epithelial cell*, (8) *the mononuclear eosinophil cell* and (9) *the large mononuclear leucocyte*. The first four of these are granular leucocytes. The first-named cell is the typical *bovine polymorph*, the cytoplasmic granules of which have eosinophilic properties even when observed in paraffin sections. The second cell is the *bovine eosinophil* which has larger eosinophilic granules than the *bovine polymorph*. The third cell would appear to be the *basophil cell* of bovine blood. The fourth cell has not been observed in the present

work, but it is possible that it is a degenerated granular cell. According to Varrier-Jones it was extremely rare; was 5–9 $\mu$ in diameter; was oval in shape and on rare occasions round; the cytoplasm was stained a faint blue throughout, but was filled with very fine mauve granules, except in the region of the nucleus.

In the present work the above four types of cells could not be distinguished from each other owing to the fact that the Sudan Black method was used, which stains all the granules in the cytoplasm a dark brown colour. A limited number of smears were stained by Leishman's method alone or by means of carbol chromotrope, and thus eosinophils were occasionally demonstrated. No attempt, however, was made by such methods to determine the frequency of occurrence of these cells in milk. Nevertheless, the fact that they have been observed in large numbers in the acini of stained sections of udder tissue (14) shows that they may occur in milk.

The other cells described by Varrier-Jones, using Jenner's stain, resemble closely in morphology and in staining properties the non-granular cells stained by the Sudan Black-Leishman method. One of these cells is the *lymphocyte*. This cell stains blue, the cytoplasm being paler in colour than the nucleus and appearing to one side of the latter or as a narrow ring around it. In the majority of specimens examined in the present investigation only 6% or less of the cells were lymphocytes. In a few instances, however, the proportion amounted to 20%. The lymphocytes were, as a rule, about 8 $\mu$ in diameter, but varied from 5 to 9 $\mu$ (see Fig. 1). It should be noted that Varrier-Jones found them to be from 2 to 4 $\mu$ in diameter.

*The red blood corpuscle* was found by Varrier-Jones to occur only in small numbers, one or two, in the deposit from 10 ml. of normal milk. It is therefore unlikely that they would be observed in a smear from 0·01 ml. of normal milk. In the present work they were found only in blood-stained samples of milk.

*The large epithelial cell*, described by Varrier-Jones as measuring up to 20 $\mu$ in diameter and containing fat globules in the cytoplasm, is the vacuolated epithelial cell. Varrier-Jones states that these large epithelial cells are present in almost all samples of milk. However, in the present work they were frequently absent from samples, although, if present, generally occurred in large numbers.

The term *mononuclear eosinophil* is used by Varrier-Jones for two types of cells, one with vacuolated cytoplasm, the other with unvacuolated cytoplasm. Occasionally vacuolated cells with eosinophilic cytoplasm have been observed in the present investigation in association with vacuolated cells referred to in the preceding paragraph. In his description of the unvacuolated mononuclear eosinophil, Varrier-Jones refers to a figure in which he shows one unvacuolated cell of about 9–10 $\mu$ with eosinophilic cytoplasm. This resembles a plasma cell. In the present work such cells have not been observed in milk samples.

*The large mononuclear leucocyte*, described by Varrier-Jones, is not a leucocyte, but the non-vacuolated epithelial cell which is found in practically all milk smears.

From the results of the present investigation, it is possible to assess the value of the findings of some of the earlier workers. For instance, Pennington & Roberts (1) were in error in stating that 75% of cows show pus in milk at the end of lactation, for although at this stage large numbers of cells are as a rule present, these are mainly non-vacuolated or vacuolated epithelial cells, and not pus cells. Revis (2) was not correct in his assumption that the presence of large numbers of leucocytes in milk was of no pathological significance.

The failure of Hewlett *et al.* (3, 4) to find polymorphs in milk appears to be due to the fact that they treated the milk with formaldehyde and did not realize that this caused an apparent reduction in the size of these cells. The cells which according to Savage (5) are large leucocytes and constitute 70% of the cells in milk from cows at the end of lactation are not leucocytes but vacuolated and non-vacuolated epithelial cells. Scannell's observation (6) that a few leucocytes in milk do not constitute pus is in agreement with the present work; this also applies to the findings of Breed (7) that milk contains leucocytes and epithelial cells. The statement of Varrier-Jones (8) that polymorphs must be dead before pus is constituted is not in accordance with fact. Bourgeois's statement (9) that multinucleate cells and mononucleate cells are present in the ratio of approximately 1:1 in normal milk may be true, but this ratio also occurs in certain stages of mastitis (14).

SUMMARY

Using a differential staining technique, total and differential cell counts were carried out on over 5000 milk samples.

It was found that the cells in both normal and abnormal milk fell into three groups:

(1) Granular leucocytes, including polymorphs, eosinophils and probably basophils.

(2) Lymphocytes.

(3) Epithelial cells (vacuolated and non-vacuolated).

The findings of some earlier workers are discussed.

We wish to thank Prof. Cappell for his help and encouragement during this work, which was part of a three-year programme of research on Bovine Mastitis. The work was made possible by grants from the Agricultural Research Council.

REFERENCES

(1) Pennington, M. E. & Roberts, E. L. (1908). *J. infect. Dis.* **5**, 92.
(2) Revis, C. (1908). *J. R. Inst. publ. Hlth*, **14**, 734.
(3) Hewlett, R. T., Villar, S. & Revis, C. (1909). *J. Hyg., Camb.*, **9**, 271.
(4) Hewlett, R. T., Villar, S. & Revis, C. (1910). *J. Hyg., Camb.*, **10**, 56.
(5) Savage, W. G. (1910). *J. R. Inst. publ. Hlth*, **18**, 65.
(6) Scannell, J. J. (1912). *Amer. J. publ. Hlth*, **2**, 962.
(7) Breed, R. S. (1914). *J. infect, Dis.* **1**, 93.
(8) Varrier-Jones, P. C. (1924). *Lancet*, no. 207, p. 537.
(9) Bourgeois, E. (1927). *Lait*, **7**, 525.
(10) Holm, G. C. (1934). *J. Amer. vet. med. Ass.* **85**, 735.
(11) Zlotnik, I. (1947). *J. comp. Path.* **57**, 196.
(12) Prescott, S. C. & Breed, R. S. (1910). *J. infect. Dis.* **7**, 632.
(13) Sheehan, H. L. & Storey, G. W. (1947). *J. Path. Bact.* **59**, 336.
(14) Blackburn, P. S. In preparation.
(15) McFarlane, D. (1946). Personal communication.
(16) Spencer, G. R. & McNutt, S. H. (1950). *Amer. J. vet. Res.* **11**, 188.

(*MS. received for publication* 6 *May* 1953)

637·133·3:577·153·3

# 535. REACTIVATION OF MILK PHOSPHATASE FOLLOWING HEAT TREATMENT. III

By R. C. WRIGHT and J. TRAMER
*United Dairies Research Laboratories, London*

In a previous paper(1) it was stated that reactivation of milk phosphatase occurred with certain raw milks when pasteurized at 161° F. in a small H.T.S.T. plant. That is to say, milks giving a negative phosphatase test immediately after pasteurizing gave a positive test after storage for 18 hr. at 26° C.

An account of the work using this small-scale plant is set out in this paper, with a brief description of the plant used.

## H.T.S.T. PLANT

This is a 50 gal. per hour plate heat exchanger type H.T.A. manufactured by A.P.V. Co. Ltd. and designed to heat milk from 40–50° F. to 161° F. and cool to 40° F. by 82% regeneration filtering at 123°/127° F., 12:1 hot water and 6:1 chilled water at 33° F. The tubular holding section is equipped with a flow diversion valve at some distance from the regenerator inlet, and for this reason the holding time was determined, using the Dummett & Mongar nickel chloride injection method, from the heater outlet to regenerator inlet. The effective holding time was 23–24 sec. The flow was found to be streamline, and thus the holder had a poor efficiency—approx. 60%. With a hot water/milk ratio of 12:1 temperature control was satisfactory, the pasteurizing temperature fluctuating by less than 0·25° F. Response to change in the control setting was rapid.

Because of the streamline flow and the mixing possible in the filter chamber, the time of sampling when changing from one milk supply to another was adjusted so as to give minimum admixture as predetermined by tests on milk after passage through the plant immediately following on water.

## PROCEDURE

Since the addition of penicillin to the raw milk, as in our capillary experiments(2), required large quantities of this antibiotic, and since it was soon found that no significant increase in reactivation took place when pasteurized milks were stored for longer than 18 hr., the use of penicillin was discontinued in these plant experiments. To avoid undue interference of bacterial growth during this storage period, the storage temperature was lowered from 30 to 26° C., previous experience with capillaries having shown that this made little difference to the level of reactivation.

Pasteurized milks which developed a positive phosphatase reaction after storage for 18 hr. at 26° C. were laboratory pasteurized (145° F. for 30 min.) and retested. Since all known bacterial phosphatases resist laboratory pasteurization when examined in milk, the absence of any phosphatase reaction in these samples after laboratory pasteurization was taken to indicate reactivation.

The Tramer & Wight(3) modification of the Aschaffenburg & Mullen test, the A/M test, was used (1 ml. milk being added to 5 ml. buffer substrate), and throughout the results are expressed as p.p.m. chlorine, since the permanent colour standards used in the micro-phosphatase test(2) are calibrated in these units.

*Experiments with bulk raw milk*

In our earlier experiments using the capillary technique, ageing of the raw milk led to increased reactivation, and so plant experiments were conducted on bulk raw milk immediately on arrival and after suitable ageing. The milks used were taken from road and rail tanks on arrival in London from a number of country creameries. Each milk was pasteurized at 161, 170 and 180° F., and samples taken from the cooler exit corresponding to each of these temperatures were stored at 26° C. for 18 hr.

No reactivation was obtained with any of these milks, either fresh or aged, after pasteurization at 161° F. Similarly, no reactivation was obtained when the temperature was raised to 170° F., but at 180° F. slight reactivation took place with some of the aged milks. None of the fresh milks showed any reactivation after heat treatment at 180° F.

Each of these milks was examined by the capillary technique, heating being carried out in a water-bath at the above three temperatures for 30 sec. Some of the aged samples gave good reactivation even after heat treatment at 161° F.

This failure of commercial samples of bulk raw milk to reactivate after pasteurization at 161° F. in the small-scale plant, confirmed our previous experience with commercial samples of H.T.S.T. pasteurized milk, which had not shown any reactivation. A direct examination was then made over a period of 3 weeks of samples of H.T.S.T. pasteurized milk taken from the cooler exit of a commercial plant. The raw-milk supply to this plant was almost entirely bulk supplies from road or rail tanks. No reactivation was observed in any of these samples.

*Experiments with milk from individual farmers*

Individual farm supplies were also examined. These milks arrived at a London processing depot by road between noon and 3 p.m. each day and consisted of that day's a.m. milking and the previous day's p.m. milking. 20 gal. of milk (bulk of two churns) from each supply were examined after pasteurization at 161, 170 and 180° F. Capillary tests were also made at these temperatures.

Of four farm supplies so examined, two showed reactivation after pasteurization on the small-scale plant at all three temperatures, and the results were confirmed on further samples of these two supplies taken 2 days later.

One of these two supplies was then used for subsequent work, and it was not until this supply failed to reactivate after pasteurization at 161° F. that an examination was made of further farm supplies in the hope that a suitable milk would be found and enable us to complete certain experiments. In this further investigation some twenty different farm milks were examined, but none showed reactivation after pasteurization at any of the three temperatures. These latter tests were made during April–May, whereas the original examination was made in January.

Reactivation following pasteurization at 161° F. of the bulk supply from this particular farm occurred when the milk was first examined during January, and continued until the middle of March. From that time onwards no reactivation has been obtained after plant pasteurization at 161° F. and, moreover, the reactivation obtained following heat treatment in capillaries at 203° F. of individual cow samples taken in July was, with a few marked exceptions, considerably lower than that obtained from these samples on the two previous visits (February and March). Some seasonal influence may thus exist.

*Experiments with a 'reactivable' farm milk*

The milk used in these experiments came from a pedigree herd of Friesians on a farm in Buckinghamshire. When this work was carried out seven churns of a.m. milk and four churns of p.m. milk were received each day, amounting to 100–110 gal. in all.

Visits to the farm were made, and it was found that the cows were left out at night as well as during the day. Rations were normal, milking was by machine, and the milk cooled by an immersion cooler. There were forty cows in the herd.

Typical results obtained from single churns of this supply are given in Tables 1 and 2. Pasteurization was carried out at 161° F., each churn being tipped into a small tipping tank and the milk only allowed to flow into the balance tank when the latter was just empty. In this way mixing of milk from different churns was minimized. Samples were taken at the cooler exit, with the usual aseptic precautions.

Table 1

| | 2 hr. A/M phosphatase test on samples stored for 18 hr. at 26° C. | | | | | | |
|---|---|---|---|---|---|---|---|
| | Plant samples 161° F. | | Capillary samples | | Resazurin disk reading | Chemical analysis | |
| Churn | Direct | After lab. pastn. | 161° F. for 30 sec. | 203° F. for 30 sec. | raw milk 1 hr. | Fat | S.N.F. |
| | | | a.m. milk | | | | |
| A | 0·1+ | Nil | 0·1− | 0·4− | 5½ | 2·65 | 8·50 |
| B | 0·1− | Nil | 0·1− | 0·3− | 5½ | 2·85 | 8·51 |
| C | 0·2 | Nil | 0·1− | 0·3 | 5½ | 3·55 | 8·60 |
| D | 0·15+ | Nil | 0·1− | 0·45 | 6 | 3·50 | 8·47 |
| E | 0·3+ | Nil | 0·1− | 0·5+ | 6 | 3·05 | 8·73 |
| F | 0·15 | Nil | 0·1− | 0·45 | 6 | 2·85 | 8·69 |
| G | 0·2 | Nil | 0·1− | 0·4 | 5 | 2·90 | 8·50 |
| | | | p.m. milk | | | | |
| H | Trace | Nil | 0·1− | 0·5 | 2 | 3·90 | 8·75 |
| I | 0·15− | Nil | 0·1 | 0·4− | 5 | 5·00 | 8·82 |
| K | 0·1+ | Nil | 0·1− | 0·4+ | 4 | 4·50 | 8·54 |
| L | 0·2− | Nil | 0·1+ | 0·35 | 5 | 4·65 | 8·60 |

Samples tested after 18 hr. cold storage gave a negative phosphatase test.

Table 2

| | 2 hr. A/M test | | 24 hr. Kay-Graham test (L.B.U.) | | | Plate-count milk agar |
|---|---|---|---|---|---|---|
| Churn | Direct | After L.P. | Direct | After L.P. | Control | 37° C. |
| E | 0·3+ | Nil | 5·0 | <1·5 | <1·5 | 11,000 |
| G | 0·2 | Nil | 3·5 | <1·5 | <1·5 | 67,000 |

These results show the considerable variation in reactivation from churn to churn. Apart from one churn (H) the milks were all of good bacteriological quality, and chemical analysis shows no correlation of either fat or solids-not-fat with reactivation. The absence of any phosphatase reaction after laboratory pasteurization (L.P.) of a milk which has developed a positive reaction during storage is indicative of reactivated milk phosphatase. The low plate counts in Table 2 exclude the possibility of interference from bacteria.

It will also be seen that there is no correlation between the plant results and those obtained by the capillary method. This is referred to later.

In view of the variation encountered from individual churns, the milk from individual cows was next examined.

*Examination of single cow samples*

These were examined by the capillary method, since they could not be pasteurized on the small-scale plant. An 8 oz. sample of the milk was taken from the pail after completion of milking of each cow. These were taken from the a.m. milking and tested at the laboratory on the same day.

The results showed that no reactivation was obtained after heat treatment in capillaries at 161° F. for 30 sec. Considerable reactivation took place, on the other hand, after heat treatment at 203° F. (95° C.) for 30 sec., which had been found to give maximum reactivation. As with the churn samples, wide variations were found, the results varying from only a trace to 0·5 p.p.m. 'chlorine' after 20–24 hr. storage at 26° C. To confirm these variations some of the raw milks were shocked by the capillary method at 203° F. for 30 sec. after 2 and 7 days cold storage and tested for phosphatase after storage for 20 hr. at 26° C. These results, together with those obtained on the same milks when first tested, are given in Table 3.

Table 3

| | | Number of days cold storage of raw milk | | |
|---|---|---|---|---|
| | Cow | 0 | 2 | 7 |
| Good reactivation: | Belle 4 | 0·4 | 0·4+ | 0·3+ |
| | Titania 13 | 0·4+ | 0·4− | 0·4− |
| | Becasse 11 | 0·5− | 0·4 | 0·4 |
| | Becasse 10 | 0·5− | 0·4+ | 0·4 |
| | Madge 9 | 0·5 | 0·4− | 0·3 |
| Average reactivation: | Wendy 2 | 0·25 | 0·3− | 0·3 |
| | Lydia 13 | 0·2+ | 0·25 | 0·3− |
| Poor reactivation: | Madge 6 | 0·1− | 0·1− | 0·1− |
| | Lux 3 | Trace | Trace | 0·1+ |
| | Fluffy 10 | 0·1− | 0·1− | 0·1− |
| | Basket 9 | 0·1− | 0·1− | 0·1− |

The figures in Table 3 show that the variations were not affected by cold storage of the raw milk prior to shock treatment and thus further confirm that they are genuine.

A second visit a fortnight later was paid to the farm and samples again taken from the complete milking of each cow. This time, however, p.m. milks were sampled and the laboratory tests carried out on the following morning, after overnight cold storage. Many of these samples reactivated after heat treatment at 161° F. for 30 sec., but the main interest lay in a comparison of the results obtained after heat treatment at 203° F. for 30 sec. on the two separate occasions. A summary of these is shown in Table 4.

Table 4

| | | 2nd farm visit (11. iii. 53) | | |
|---|---|---|---|---|
| Reactivation level | 1st farm visit (24. ii. 53) | High | Medium | Low |
| High | 11 | 8 | 1 | 2 |
| Medium | 13 | 1 | 8 | 4 |
| Low | 16 | 3 | 1 | 12 |

The figures in this table represent the number of cows falling into each of the three arbitrary reactivation categories on the two visits. For example, of the eleven cows giving high reactivation on the first visit, eight fell in the same category on the second visit and similarly of the sixteen cows giving low reactivation on the first visit, twelve fell in

the same category on the second visit. Thus the variation encountered is considered to be a real one.

Both previously and at this time attempts were made to establish the cause of this variation, but without success. Several methods of studying the SH groups of milk have been published, and in view of the influence of reducing conditions on reactivation, some of these methods (4, 5, 6) were applied to milk from this particular farm. No significant differences were found between those milks giving good reactivation and others giving poor reactivation.

*Comparison of reactivation following plant and capillary heat treatment*

In much of the work done on reactivation following heat treatment on the small-scale plant, the milks were also treated by the capillary method using an immersion time in a water-bath of 30 sec. It was found that the relationship between the results obtained by the two methods was very variable, as will be seen from Table 5, which shows the reactivation developing after storage for 18–24 hr. at 26° C., following heat treatment at three different temperatures.

Table 5

| Milk no. | 161° F. | | 170° F. | | 180° F. | |
|---|---|---|---|---|---|---|
| | Plant | Capillary | Plant | Capillary | Plant | Capillary |
| 1 | 0·2+ | 0·1− | 0·3+ | 0·1+ | 0·5+ | 0·3+ |
| 2 | 0·2+ | Trace | 0·3+ | Trace | 0·4+ | 0·1− |
| 3 | 0·35 | 0·5− | 0·3 | 0·5− | 0·35 | 0·4+ |
| 4 | Trace | 0·3 | 0·25 | 0·3+ | 0·4+ | 0·35 |
| 5 | 0·1 | 0·1− | 0·1+ | 0·1− | 0·35 | 0·3− |
| 6 | Trace | Trace | 0·1 | 0·2 | 0·3+ | 0·3− |
| 7 | 0·1− | 0·3 | 0·1− | 0·35 | 0·1+ | 0·3− |
| 8 | 0·2+ | Nil | 0·3+ | 0·2 | 0·5+ | 0·4 |
| 9 | 0·1− | 0·1+ | 0·1+ | 0·1− | 0·3+ | 0·4 |
| 10 | 0·2 | Nil | 0·3 | Nil | 0·5+ | 0·1+ |
| 11 | 0·35 | 0·7 | 0·3 | 0·7 | 0·35 | 0·65 |
| 12 | Nil | 0·4 | 0·25 | 0·4+ | 0·4+ | 0·4+ |

Of the twelve samples given in this Table, nine were samples of bulk churn milk from the same farm taken on different days. Whatever the explanation it is clear that testing milks by the capillary method gives no indication of the reactivation likely to develop in those milks when pasteurized on the plant.

Table 5 also illustrates the variable relationship between the results after heat treatment at the three temperatures with both methods, even though nine of the twelve samples all came from the same farm (at different times). Whilst some milks gave poor reactivation at 161° F., and good reactivation at 180° F., others gave good reactivation at all three temperatures.

*Effect of cold storage of pateurized milk upon subsequent reactivation*

In examining the milk from the Buckinghamshire farm previously mentioned, it was noted that samples of plant-pasteurized milk gave less reactivation when tested after storage following refrigeration than when tested after storage immediately following processing. Since many samples of pasteurized milk taken in this country are either cold stored before carrying out the phosphatase test, or are drawn from pasteurized milk which may have been cold stored for some hours, it was decided to investigate this point more fully.

Over twenty different samples of milk from this farm were examined, and in each case cold storage of the pasteurized milk reduced the level of reactivation produced after subsequent storage at 26° C. After pasteurization at 161° F., followed by cold storage for 2 days, no sample developed a phosphatase reaction in excess of 0·15 p.p.m.'chlorine' (equivalent to 2·3 L.B.U.). At higher temperatures of pasteurization, namely, 170 and 180° F., the same effect was observed, although some of the results suggested that the ability of the milk to reactivate is not affected by cold storage as much as it is after pasteurization at 161° F.

In this connexion, the ability of the samples of Uperized milk previously reported (2) to reactivate was not affected by cold storage, for such milks gave the same phosphatase value after 3 weeks at 26° C., irrespective of prior cold storage up to 3 weeks. Similarly, repeated tests have shown that the developed or reactivated phosphatase, irrespective of the method of heating used, is not affected by subsequent cold storage. For instance, one sample which had been stored in the refrigerator for 10 months, following 3 weeks at 26° C., gave a phosphatase value identical with that (23 L.B.U.) which was obtained when the sample was tested originally, i.e. immediately following 3 weeks' storage at 26° C.

Tests carried out on milks shock-treated by the capillary method showed that cold storage did not affect the subsequent reactivation, irrespective of the temperature (80–140° C.), and thus point to another difference resulting from the two methods of heat treatment.

*Effect of cold storage of raw milk upon subsequent reactivation*

As already shown, no reactivation took place when commercial bulk raw milk supplies, as received in London, were pasteurized at 161° F. in the small-scale plant. On the other hand, certain farm supplies gave rise to reactivation after similar pasteurization, and therefore, since raw milk from road or rail tanks is stored at 40–45° F. for at least several hours longer than these farm supplies, it was decided to investigate also the effect of cold storage of the raw milk upon subsequent reactivation.

Unfortunately, only a few observations have been made on this aspect, since by the time it was possible to carry out further tests, the farm milk previously used failed to reactivate. The few observations made, however, suggest that cold storage of the raw milk results in less reactivation being produced after plant pasteurization at 161° F. and subsequent storage.

Using the capillary method, we have on many occasions tested raw milks before and after cold storage and have rarely observed any marked effect of cold storage. This is borne out by the results given in Table 3.

*Effect of pasteurizing in the absence of oxygen*

It has already been shown (1) that when raw milk is shocked in capillaries in the absence of oxygen, the reactivation developed on subsequent storage is considerably greater than that developed after shock treatment in air. The only exceptions reported were aged raw milks and certain other raw milks which, in the presence of air, were found to be highly susceptible to reactivation. In these cases no increase was found when shock treatment in capillaries took place in an atmosphere of nitrogen.

A few experiments have been made using the small-scale plant and pasteurizing in the presence of nitrogen. The raw milk used was from the Buckinghamshire farm and was

allowed to flow from the tipping tank to the balance tank, and then nitrogen was bubbled through the milk in both tanks with the aid of gas distribution tubes (sintered glass) for 30 min. approximately. The pump was then switched on, thus passing the milk forward into the clean but empty plant. Samples were not taken from the cooler exit until after about 5 min. running, in order to minimize the possible presence of air, before raising the temperature and taking further samples. A duplicate bulk of the untreated milk was similarly pasteurized immediately after the nitrogen-treated milk. Table 6 shows the results obtained.

Table 6

| | Pasteurizing temperature | | | | | |
|---|---|---|---|---|---|---|
| | 161° F. | | 170° F. | | 180° F. | |
| Raw milk | Plant | Capillaries | Plant | Capillaries | Plant | Capillaries |
| Fresh | Nil | 0·4 | 0·25 | 0·4+ | 0·4+ | 0·4+ |
| Fresh + $N_2$ | 0·3− | 0·6 | 0·4+ | 0·8− | 0·5 | 0·8+ |
| Cold stored | Nil | 0·25 | Nil | — | 0·2 | 0·5 |
| Cold stored + $N_2$ | Nil | 0·6 | 0·1 | 0·55 | 0·3 | 0·7 |

These figures show that whilst pasteurizing in the absence of oxygen on the plant leads to increased reactivation in the case of fresh milk, this increase is less marked in the same milk after cold storage. At 161° F. no reactivation occurred in the cold-stored milk even in the absence of oxygen.

*Effect of shortened holding time*

In much of the work carried out on the small-scale plant, pasteurizing was conducted at three temperatures namely, 161, 170 and 180° F., and some of the results obtained appear in Tables 5 and 6. In general, it has been found that greater reactivation is produced as the pasteurizing temperature is raised.

It was of interest to determine the effect of shortening the plant holding time upon subsequent reactivation and the plant was modified accordingly. The effective holding time under normal running was 23–24 sec. The short holding time used was 6 sec., and under these modified conditions a positive phosphatase test was given at 161° F. due to residual phosphatase. At a pasteurizing temperature of 165° F. the phosphatase test, carried out immediately after pasteurizing, fell to 2·3 L.B.U. approximately, and at 167·5° F. and upwards, the normal value of 1·5 L.B.U. was obtained.

Because of this the results in Table 7 give the reactivation developed after 18–24 hr. at 26° C. following pasteurization at 170 and 180° F. with both normal and short holding

Table 7

| | 161° F. | | 170° F. | | 180° F. | |
|---|---|---|---|---|---|---|
| Milk no. | N | S | N | S | N | S |
| 1 | 0·1 | — | 0·1+ | 0·5− | 0·35 | 0·6− |
| 2 | 0·1− | — | 0·1 | 0·1 | 0·3+ | 0·1 |
| 3 | Trace | — | Trace | 0·15 | 0·25 | 0·3+ |
| 4 | 0·1− | — | 0·1 | 0·25 | 0·35 | 0·4− |
| 5 | 0·1+ | — | 0·1+ | 0·15 | 0·3+ | 0·3 |
| 6 | 0·1+ | — | 0·15 | 0·3+ | 0·4 | 0·55 |
| 7 | 0·1− | — | 0·1− | 0·1− | 0·2+ | 0·1− |
| 8 | 0·1− | — | 0·2 | 0·1+ | 0·4− | 0·25 |
| 9 | 0·1− | — | 0·1− | 0·1 | 0·15+ | 0·2 |

N = normal holding time (23–24 sec.). S = short holding time (6 sec.).

time, together with that developed after pasteurizing with normal holding time at 161° F. In each test a bulk of raw milk was divided into two portions before carrying out the comparative testing.

It will be seen that pasteurizing at 170 or 180° F. with a short holding time may well give rise to greater reactivation than with a long holding time, though the figures are not consistent. Evidence was obtained that cold storage had a greater effect upon the reactivation, developed on subsequent storage, with milks pasteurized using a short holding time than in those pasteurized using the normal holding time. The figures illustrate, however, that reactivation increased with rise in temperature of pasteurization. Isolated observations have been made of the reactivation developing after pasteurizing at 203° F., and in each case this has been greater than that developing after pasteurizing the same milk at lower temperatures, and therefore partially confirms our previous findings, using the capillary method, that maximum reactivation occurred after heating to 95° C. (203° F.).

*Experiments with a Danish pasteurizer*

A few experiments have been carried out using a Danish pasteurizer and flashing to 180° F. upwards. Reactivation has been found to occur after subsequent storage though at a lower level than after pasteurizing a duplicate bulk of milk in the small-scale H.T.S.T. plant at similar temperatures with the normal holding time. These results thus confirm the high phosphatase results reported earlier(2), found in milks pasteurized by this method from a country creamery.

*Experiments on milk flash pasteurized by plate type A.P.V. plant*

Tests have been carried out over a period of several months on milk flash pasteurized to 180° F. approx. in an A.P.V. plant at a country creamery. Whilst samples of this milk preserved with $HgCl_2$ (concentration 1 in 10,000) have given a normal phosphatase value, duplicate samples stored at atmospheric temperature for 1–2 days have developed a slight phosphatase reaction which in some cases was well over 2·3 L.B.U. This positive reaction was not due to phosphatase of bacterial origin. It is thus shown that reactivation may occur in milk flash pasteurized on this type of plant.

*Effect of double-heat treatment*

A few observations were made on the effect of repasteurization, using the normal holding time, and the results are shown in Table 8.

Table 8

| Milk no. | 161 once only | Heat treatment given in °F. 161 + 180 | 180 once only | 180 + 161 | 180 + 180 | 180 + 145/15 min. |
|---|---|---|---|---|---|---|
| 1 | 0·1 | 0·2+ | 0·35 | 0·3 | 0·3 | 0·1⁻ |
| 2 | Trace | 0·15 | 0·25 | 0·2 | 0·25 | — |
| 3 | 0·1⁻ | 0·2⁻ | 0·35 | 0·25 | 0·25 | — |

These results confirm our previous findings, both on the plant and by capillaries. It was interesting to find that re-heating for 15 min. at 145° F. a milk pasteurized at 180° F. led to a very marked fall in reactivation, for this agrees with our findings on the effect of reheating by the capillary method(1).

DISCUSSION

As far as we are aware, the only other published evidence on the reactivation of the alkaline phosphatase of milk following heat treatment is that of Fuchs (7), who confirmed our original findings upon Uperized milk. The work of this author has also been referred to by Mohler (8).

There have been many reports of pasteurized cream giving high phosphatase values, though in some of these it is not clear whether this high value was found immediately following pasteurization or at some later stage. Brown & Elliker (9) reported many cases of pasteurized cream developing a positive phosphatase test on storage, even at temperatures as low as 4·4° C. They also observed that this increase in phosphatase value did not occur when cream was pasteurized by the holder process. Burgwald (10), in a review on the phosphatase test, refers to Fay & Barber, who observed that these high phosphatase values obtained on pasteurized cream might be due to 'reversible coagulation of the enzyme'. In a later paper, however, Barber (11), in discussing these cases, put forward a bacteriological explanation, but admitted that large numbers of bacteria (48–434 $\times 10^6$/ml.) were necessary before sterilized cream, inoculated and stored at low temperatures, showed a positive phosphatase test. Much lower numbers ($2 \times 10^6$/ml.) were found to be necessary in pasteurized cream similarly inoculated and stored at low temperatures, and even the uninoculated pasteurized cream itself gave a positive phosphatase result after longer storage at these low temperatures. In Holland an investigation into the use of vacreators has been made, and in its report (12) the Committee refers to the occurrence in pasteurized cream of positive phosphatase values which are presumed to be due to constructional defects in the pasteurizing plant, though it is admitted that such creams gave a negative peroxidase reaction. Since this enzyme is more heat-stable than phosphatase this seems an unlikely explanation, although it is possible if the peroxidase test reagents are not sufficiently sensitive, for they can vary considerably in this respect. Posthumus (13) has studied this problem in some detail, and has suggested that these positive reactions developing in pasteurized cream are due to failure of the heat treatment given to penetrate into fat conglomerates sufficiently to destroy phosphatase held within these fat globules. Though such creams give a negative phosphatase reaction when tested immediately after pasteurization, the development of a positive reaction on subsequent storage is considered to be due to diffusion of this undestroyed phosphatase into the serum. A similar explanation has been put forward by Wiley, Newman & Whitehead (14), who investigated the occurrence of positive phosphatase results in butter manufactured from pasteurized cream. These authors concluded that cream pasteurized by the H.T.S.T. process contains residual 'bound' phosphatase which is liberated in the presence of salt. They also stated that such creams developed a positive phosphatase reaction in the presence of sugar and also when dried *in vacuo*. Ritter (15), in his review on the phosphatase test, refers to the high phosphatase values reported by various authors following storage of properly pasteurized cream. He states that these high values only occur in cream pasteurized by H.T.S.T. or flash processes, and that the temperature to which the cream is heated is not critical. In his own experience, high values have been obtained from cream heated to temperatures varying from 72 to 90° C. (162–194° F.), but points out that the holding time is of greater importance, the shorter the time of heating the higher the values obtained. In his opinion the explanation of these results must await further

evidence, but they may be due to regeneration of phosphatase like that known to occur with peroxidase.

The suggestion that the development of a positive phosphatase reaction in pasteurized cream is due to failure of the heat treatment to inactivate the enzyme protected by fat clusters is, in our opinion, untenable. Posthumus, himself, found that this developed phosphatase reaction tended to be greater with creams pasteurized at high temperatures (85–95° C.) than at lower temperatures (75–85° C.). He also observed that this reaction after storage at 45° C. was much less than that developed after storage at 30–37° C., which is not what one would expect to happen if the undestroyed enzyme diffuses out during storage. Furthermore, if this theory is correct, one would expect diffusion of such 'protected' phosphatase into the buffer substrate to occur during the phosphatase test, but there is no evidence that this takes place. Again, it has been said that an increase in phosphatase reaction only occurs with cream pasteurized by H.T.S.T. or flash processes, the inference being that the holder process of pasteurizing is sufficient to inactivate the enzyme completely. Whilst the storage of either cream or milk pasteurized by the holder process does not give rise to any increased phosphatase activity, nevertheless, if such cream or milk is subsequently flash-heated to a higher temperature, a phosphatase reaction then develops on storage. Finally we have shown that skim milk when shocked to a high temperature develops a phosphatase reaction on storage.

It is not surprising that this increase in phosphatase reaction on storage has been also attributed to bacterial phosphatase. It is the common experience of those who have worked on this aspect that the number of organisms required to bring about any increase in the phosphatase reaction of milk or cream is at least several millions per ml. In our work with milk using time/temperature conditions insufficient to bring about sterility, it has been found that milks showing this increased phosphatase reaction had counts very much lower than the above figure. Moreover, it has been shown (2) that bacterial phosphatases are readily distinguishable from the alkaline phosphatase of milk, and throughout this investigation laboratory pasteurization (145° F./30 min.) of milks showing a positive phosphatase reaction after storage has been used as a routine test. When necessary the Kay-Graham test and the effect of change of pH of the buffer substrate have been used as confirmatory tests. Moreover, this development of a phosphatase reaction after storage occurs in milk submitted to a wide range of time/temperature combinations in excess of those necessary to sterilize the milk.

In considering these various reports and, in particular, the experimental results of Posthumus, it seems to us that many of these cases of pasteurized creams giving rise to positive phosphatase tests on storage can be explained as being due to reactivation. Examination of commercial samples of cream has shown that a positive phosphatase reaction may develop in 'double' cream (48% butter-fat) during storage at 26° C., and as with milk this was not of bacterial origin.

The reactivation of milk peroxidase was reported by Fischer and others in Germany in milk heated to 85–87° C. (185–189° F.), and it is of interest to find that Paschke (16) suggests that the phosphatase test should be used on these heated milks giving a positive peroxidase test in order to decide whether the milk has been contaminated with raw milk after pasteurization. Clearly the value of such a procedure is greatly diminished in view of the reactivation of phosphatase which may well occur in such milks. It is also of interest that reactivated peroxidase differs in certain respects from normal milk per-

oxidase, whereas we have been unable to detect any such differences with milk phosphatase.

In a recent paper (17) this author has studied the possibility of the occurrence of reactivation of phosphatase in milks pasteurized at 68–74° C. for a holding time of 40 sec. He was unable to detect reactivation, but this is not surprising in view of the storage conditions used, namely, either 2–3 hr. at 37° C. or 3 days at 15–20° C., the latter in the presence of preservative. In our experience 2–3 hr. at 37° C. is too short a time for the satisfactory development of reactivation, and further, we have never found reactivation to occur in the presence of preservative ($HgCl_2$). Furthermore, he did not investigate the possible reactivation of phosphatase after flash pasteurization at 85–87° C. (185–189° F.), which is a more favourable range for its occurrence. Also this temperature range is of special interest in view of his earlier suggestion that a positive phosphatase reaction given by a milk after such heat treatment should be taken as indicative of an admixture of raw milk.

This reactivation of milk phosphatase must be considered in the light of the present methods of pasteurizing, particularly those which have legal status in this country. From the work reported in this paper it would appear that pasteurization by the usual H.T.S.T. process (161° F. for 15 sec.) is unlikely to result in any reactivation occurring with bulk supplies of raw milk as received in London and other large towns. On the other hand, the pasteurization by this process of fresh raw milk from farms might lead to some reactivation and thus cause a serious complication in the application of the official phosphatase test. However, as any reactivation usually seems to require at least several hours' storage at temperatures above 70° F. before it becomes evident, it may be considered that there is little chance of this occurring in practice, and a further safeguard in this connexion is the fact that cold storage of the pasteurized milk lessens the amount of reactivation taking place on subsequent storage at higher temperatures. Since in this country pasteurized milk must be immediately cooled to a temperature not more than 50° F., and, in fact, is usually coooled to 40–42° F., the real danger of reactivation occurring would be in failure to keep the milk at such low temperatures. This might well happen if samples of pasteurized milk, particularly those taken soon after pasteurizing, were allowed to warm up for a few hours prior to testing, either in transit to or in the laboratory where testing is conducted. The possibility of reactivation occurring in pasteurized milk from bottles on the roundsman's van would seem to us a very real one in hot summer weather when fresh raw milk from farms is pasteurized and sent out on the roundsman's van soon after bottling.

The effect of pasteurizing at temperatures higher than 161° F. must also be considered in view of the fact that the present legislation in this country does not preclude pasteurizing at temperatures well above 161° F. It is clear from the work we have carried out on this aspect that the possibility of reactivation occurring is much increased by such high-temperature pasteurizing. Practical evidence confirming this is found in the results of our examination of milk flash pasteurized to 180° F. approximately in a commercial plant at the present time, and similar evidence was also found some years ago in such flash-pasteurized milk from another commercial plant.

If, at any future time, it is desired to raise the pasteurizing temperature, then modifications to the present regulations governing the taking of samples would be necessary in order to overcome the difficulty which otherwise might well be brought about by reactivation.

As far as holder pasteurization (145° F. for 30 min.) is concerned, we have not at any time been able to find any evidence of the occurrence of reactivation, either in commercial samples or in laboratory samples.

Work now in progress on cream suggests that it is probable that the same considerations apply to pasteurized cream. Also, owing to the higher phosphatase content of cream, the possibility of detectable reactivation is greater, though in the absence of an official phosphatase test for cream, the problem involved is not a practical one so far. If the application of this test to cream arises, then this problem of reactivation must be taken into consideration.

## SUMMARY

1. Of a number of farm milks examined, two have been found to develop a positive phosphatase reaction following pasteurization at 161° F. in a small H.T.S.T. plant. This phosphatase was not of bacterial origin but was reactivated milk phosphatase.

2. Considerable variation in reactivation following pasteurization at 161° F. was found to occur using individual churns of the above farm milk from any one milking. Individual cow samples also showed similar variation when tested by the capillary method. No explanation of this variation is offered.

3. Commercial bulk raw milk from road or rail tanks did not develop a positive phosphatase reaction after pasteurization at 161° F. on this plant.

4. Cold storage of pasteurized milk susceptible to reactivation results in less reactivation, and some evidence was obtained that cold storage of the corresponding raw milk also results in less reactivation. No such effect has been observed either using the capillary method or with samples of Uperized milk.

5. Pasteurizing at 170 and 180° F. usually resulted in increased reactivation as compared with that obtained after pasteurization at 161° F. Shortening the holding time at these two higher temperatures sometimes led to a further increase in reactivation.

6. It is shown that no clear relationship exists between the results obtained by plant pasteurization and the capillary method.

7. It is suggested that many of the reported cases of pasteurized cream developing a positive phosphatase reaction on storage can be attributed to reactivation of the enzyme.

8. Practical implications of reactivation in relation to pasteurized milk are discussed.

The authors would like to express their thanks to Mr P. A. Hoare for operating the small-scale H.T.S.T. plant.

## REFERENCES

(1) Wright, R. C. & Tramer, J. (1953). *J. Dairy Res.* **20**, 258.
(2) Wright, R. C. & Tramer, J. (1953). *J. Dairy Res.* **20**, 177.
(3) Tramer, J. & Wight, J. (1950). *J. Dairy Res.* **17**, 194.
(4) Patton, S. & Josephson, D. V. (1949). *J. Dairy Sci.* **32**, 398.
(5) Gould, I. A. (1940). *J. Dairy Sci.* **23**, 977.
(6) Rosner, L. (1940). *J. Biol. Chem.* **132**, 657.
(7) Fuchs, A. (1953). *XIIIth Intern. Dairy Congr.*, **3**, 1018.
(8) Mohler, H. (1952). *Chimia*, **6**, 212.
(9) Brown, W. H. & Elliker, P. R. (1941). *Proc. Indiana Branch, Soc. Amer. Bact.* 14 Nov. (*J. Bact.* **43**, 118, 1942.)

(10) BURGWALD, L. H. (1941). *Int. Ass. Milk Dlrs*, **33** (14) 366.
(11) BARBER, F. W. (1942). *Milk Dlr*, **32**, 66.
(12) *Report of Committee for Expts. of the Royal Neth. Dairy Fedn. Neth. Milk & Dairy Journal*, **6**, (1952), 82.
(13) POSTHUMUS, G. (1952). *Off. Organ. F.N.Z.* **44**, 458.
(14) WILEY, W. J., NEWMAN, F. S. J. & WHITEHEAD, H. R. (1941). *J. counc. Sci. Indust. Res.* **14**, 121.
(15) RITTER, W. (1952). *Milchwissenschaft*, **7**, 301.
(16) PASCHKE, B. (1952). *Milchwissenschaft*, **7**, 3.
(17) PASCHKE, B. (1953). *Milchwissenschaft*, **8**, 266.

(*MS. received for publication* 14 *September* 1953)

637.127.1:612.664.36

# 536. THE PHYSICAL PROPERTIES OF COW'S MILK AS INFLUENCED BY STAGE OF LACTATION

## THE SIZE DISTRIBUTION OF BUTTERFAT GLOBULES

By W. G. WHITTLESTONE, *Ruakura Animal Research Station, Department of Agriculture, New Zealand*

(With 1 Figure)

During the course of an experiment on the changes in composition of cow's milk, throughout each milking and throughout lactation, a series of samples was made available for the determination of fat-globule size distribution. The present paper gives some preliminary results on the trends in fat-globule size distribution for one quarter of one cow throughout a lactation. The methods used have been described elsewhere (1) in connexion with a similar study of sow's milk.

Interest in the trends in fat-globule size as a cow is being milked out, go back to early studies of milk composition (Gaines (2); Petersen, Palmer & Eccles (3)). In an earlier paper (4) the present writer has reviewed the various theories offered to explain the rise in fat content of cow's milk with the 'strippings', and has suggested that this phenomenon may be explained by assuming that the globules cluster and that the clusters are filtered out by the fine ductules. Working with perfused glands Petersen *et al.* found that fat, which should have been contained in the alveoli, could not be removed by a normal milking process. They present objections to any explanation involving the physical retardation of the fat globules in an unclustered state, and suggest some special condition which obtains in the perfused gland. It would seem that their results could be explained in terms of the clustering theory. Any physical retardation theory not involving clustering would require strippings to contain larger globules than the rest of the milk. Similarly, the idea suggested by Eisenreich & Mennicke (5) that the differences between morning and evening milk may be explained by differences in creaming rate, caused by the presence of larger globules, involves changes in the fat-globule distribution pattern.

The results presented here apply to only one quarter of one cow but show the effect of stage of milking, stage of lactation and the influence of a change from evening to morning milkings. Samples were taken during the normal machine milking of the cow, one teat-cup being connected to the sampling apparatus. When normal milking ceased the cow was given a subcutaneous injection of 30 units of Pitocin and milking resumed. Samples of 'super strippings' were thus obtained. The results of globule measurements are set out in Table 1 and Fig. 1. The samples which represent milk drawn after the injection of Pitocin (residual milk) are as follows:

| Days in milk | Sample no. |
|---|---|
| 68 | 6 |
| 101 | 7, 8, 9 |
| 115 | 8, 9, 10 |
| 142 | 4, 5, 6 |
| 178 | 5, 6, 7 |
| 226 | 5, 6, 7 |
| 255 | 4, 5 |

| Microns | 0–0·5 | 0·5–1 | 1–1·5 | 1·5–2 | 2–2·5 | 2·5–3 | 3–3·5 | 3·5–4 | 4–4·5 | 4·5–5 | 5–5·5 | 5·5–6 | 6–6·5 | 6·5–7 | 7–7·5 | 7·5–8 | 8–8·5 | 8·5–9 | 9–9·5 | 9·5–10 | 10– | No. of globules | Days in milk |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample no. | | | | | | | | | | | | | | | | | | | | | | | |
| 1 | 0·8 | 6·4 | 7·2 | 5·6 | 7·9 | 11·9 | 9·5 | 15·1 | 7·2 | 9·5 | 9·5 | 1·6 | 4·8 | 3·2 | | | | | | | | 126 | 68 |

| Sample no. | Microns 0–0·5 | 0·5–1 | 1–1·5 | 1·5–2 | 2–2·5 | 2·5–3 | 3–3·5 | 3·5–4 | 4–4·5 | 4·5–5 | 5–5·5 | 5·5–6 | 6–6·5 | 6·5–7 | 7–7·5 | 7·5–8 | 8–8·5 | 8·5–9 | 9–9·5 | 9·5–10 | 10– | No. of globules | Days in milk |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0·8 | 6·4 | 7·2 | 5·6 | 7·9 | 11·9 | 9·5 | 15·1 | 7·2 | 9·5 | 9·5 | 1·6 | 4·8 | 3·2 | — | — | — | — | — | — | — | 126 | 68 |
| 2 | 0 | 3·2 | 4·8 | 8·5 | 8·0 | 7·4 | 9·6 | 13·3 | 12·8 | 9·6 | 6·9 | 6·4 | 2·1 | 5·3 | 1·1 | 0 | 1·1 | — | — | — | — | 188 | |
| 3 | 0 | 0·6 | 5·0 | 7·2 | 10·0 | 12·2 | 15·5 | 11·1 | 11·6 | 10·5 | 6·1 | 4·4 | 3·3 | 1·7 | 1·1 | — | — | — | — | — | — | 181 | |
| 4 | 0 | 1·2 | 2·9 | 4·0 | 12·6 | 8·6 | 10·3 | 15·5 | 8·6 | 15·5 | 8·0 | 5·2 | 3·4 | 1·7 | 1·1 | 0 | 0·6 | 0·6 | — | — | — | 174 | |
| 5 | 0 | 1·0 | 3·0 | 6·0 | 8·0 | 13·1 | 15·1 | 13·1 | 11·0 | 8·0 | 8·5 | 5·0 | 3·5 | 2·5 | 0·5 | 0·5 | 0·5 | 0·5 | — | — | — | 199 | |
| 6 | 0 | 1·0 | 3·1 | 3·6 | 11·7 | 10·7 | 16·8 | 10·2 | 15·3 | 9·7 | 7·1 | 6·6 | 2·6 | 1·0 | 0·15 | — | — | — | — | — | — | 196 | |
| 1 | 3·9 | 18·8 | 11·8 | 6·3 | 9·0 | 8·2 | 5·9 | 12·5 | 7·5 | 6·7 | 3·9 | 2·7 | 1·6 | 0·4 | 0 | 0·8 | — | — | — | — | — | 255 | 101 |
| 2 | 1·1 | 2·2 | 5·5 | 4·9 | 8·2 | 15·4 | 14·8 | 14·3 | 15·4 | 9·3 | 5·5 | 2·2 | 0 | 0·5 | 0 | 0·5 | — | — | — | — | — | 182 | |
| 3 | 0 | 4·3 | 6·9 | 9·1 | 10·8 | 13·4 | 12·1 | 10·8 | 11·2 | 8·2 | 5·2 | 5·2 | 1·3 | 0·4 | 0·9 | 0·4 | — | — | — | — | — | 232 | |
| 4 | 0·9 | 3·3 | 7·7 | 10·4 | 10·4 | 10·8 | 9·0 | 12·6 | 9·9 | 8·6 | 7·7 | 4·1 | 3·2 | 0·5 | 0·9 | — | — | — | — | — | — | 222 | |
| 5 | 0·3 | 2·5 | 6·3 | 5·6 | 11·6 | 11·3 | 12·9 | 11·3 | 11·0 | 7·5 | 8·8 | 5·0 | 2·8 | 1·9 | 0·9 | 0·3 | — | — | — | — | — | 319 | |
| 6 | 0 | 3·1 | 4·2 | 11·2 | 11·2 | 9·3 | 12·7 | 14·7 | 10·0 | 10·0 | 7·3 | 2·3 | 1·2 | 1·9 | 0·8 | — | — | — | — | — | — | 259 | |
| 7 | 0 | 1·1 | 3·9 | 6·7 | 10·9 | 10·9 | 9·5 | 15·4 | 13·7 | 8·8 | 7·0 | 5·3 | 2·8 | 1·4 | 1·8 | 1·1 | — | — | — | — | — | 285 | |
| 8 | 0·4 | 0 | 2·5 | 3·6 | 4·0 | 7·9 | 15·2 | 16·2 | 11·2 | 13·0 | 12·3 | 6·1 | 3·2 | 1·8 | 1·8 | 0·4 | 0·4 | — | — | — | — | 277 | |
| 9 | 0 | 2·4 | 6·2 | 6·6 | 9·0 | 8·6 | 13·4 | 15·5 | 11·0 | 11·4 | 5·9 | 3·1 | 2·1 | 2·8 | 1·4 | 0·3 | 0·3 | — | — | — | — | 290 | |
| 1 | 2·2 | 14·1 | 14·1 | 10·8 | 10·8 | 10·3 | 7·0 | 7·6 | 8·1 | 7·0 | 5·4 | 0·5 | 0 | 1·1 | 0·5 | 0·5 | — | — | — | — | — | 185 | 115 |
| 2 | 0·6 | 8·9 | 9·5 | 11·4 | 10·0 | 16·5 | 11·4 | 7·6 | 10·8 | 1·9 | 4·4 | 3·2 | 0 | 2·5 | 0·6 | 0·6 | — | — | — | — | — | 158 | |
| 3 | 1·5 | 7·1 | 9·1 | 9·6 | 13·2 | 12·2 | 8·1 | 11·7 | 8·6 | 8·1 | 4·1 | 2·5 | 2·5 | 0·5 | 1·0 | — | — | — | — | — | — | 197 | |
| 4 | 0 | 3·4 | 8·1 | 11·9 | 14·3 | 9·1 | 12·4 | 10·1 | 12·9 | 9·1 | 4·3 | 1·9 | 0·9 | 0·9 | 0·5 | — | — | — | — | — | — | 209 | |
| 5 | 0 | 5·8 | 10·3 | 7·6 | 13·5 | 15·7 | 9·4 | 10·3 | 7·6 | 7·6 | 5·8 | 0·9 | 2·2 | 0·4 | 1·3 | 0·4 | 0·4 | 0·4 | — | — | — | 223 | |
| 6 | 0 | 4·9 | 5·4 | 8·3 | 9·3 | 8·3 | 14·7 | 13·7 | 10·8 | 8·3 | 6·4 | 2·9 | 2·9 | 3·4 | 0 | 0·5 | — | — | — | — | — | 204 | |
| 7 | Sample omitted | | | | | | | | | | | | | | | | | | | | | | |
| 8 | 0·5 | 2·5 | 7·8 | 6·9 | 7·8 | 10·8 | 11·8 | 17·1 | 9·3 | 10·3 | 5·4 | 4·9 | 1·0 | 1·5 | 0·5 | 0·5 | 1·0 | 0·5 | — | — | — | 204 | |
| 9 | 0·9 | 5·0 | 6·3 | 12·2 | 13·1 | 13·1 | 11·3 | 10·0 | 8·1 | 8·1 | 3·6 | 4·1 | 1·4 | 1·4 | 0·4 | 0·4 | 0·4 | — | — | — | — | 221 | |
| 10 | 0 | 2·6 | 4·4 | 8·4 | 11·9 | 7·5 | 16·7 | 11·9 | 8·8 | 7·9 | 7·5 | 3·5 | 3·5 | 0·9 | 1·3 | 1·8 | 0·4 | 0·4 | 0·4 | — | — | 227 | |
| 1 | 0·6 | 2·9 | 5·8 | 9·8 | 14·5 | 10·4 | 11·0 | 11·0 | 9·2 | 8·7 | 6·9 | 2·9 | 2·9 | 1·7 | 0·6 | 0 | 0·6 | 0·6 | — | — | — | 173 | 142 |
| 2 | 0 | 2·0 | 3·5 | 9·5 | 12·1 | 10·6 | 12·1 | 12·6 | 12·1 | 11·1 | 5·5 | 5·0 | 2·0 | 1·0 | 0 | 0 | 1·0 | — | — | — | — | 199 | |
| 3 | 0 | 1·1 | 7·4 | 8·0 | 14·2 | 17·0 | 11·9 | 15·9 | 6·2 | 5·7 | 5·1 | 3·4 | 1·7 | 1·1 | 1·1 | 1·1 | 0·5 | — | — | — | — | 178 | |
| 4 | 0·5 | 2·4 | 5·3 | 5·8 | 11·2 | 18·4 | 8·7 | 12·6 | 11·7 | 8·7 | 3·4 | 2·9 | 2·4 | 1·9 | 1·0 | 0 | 0·5 | 0·5 | 0·5 | 0·5 | 1·0 | 206 | |
| 5 | 0 | 2·0 | 7·1 | 8·2 | 14·3 | 18·4 | 10·7 | 13·3 | 9·7 | 6·6 | 6·1 | 2·0 | 1·5 | — | — | — | — | — | — | — | — | 196 | |
| 6 | 1·3 | 2·7 | 6·5 | 10·2 | 12·9 | 18·1 | 16·9 | 9·3 | 6·9 | 4·6 | 6·2 | 2·8 | 0·9 | 0·4 | 0 | 0 | 0·4 | 0·4 | — | — | — | 216 | |
| 1 | 0 | 7·0 | 8·3 | 8·9 | 12·7 | 12·1 | 12·7 | 14·0 | 8·9 | 5·7 | 1·9 | 1·3 | 3·2 | 0·6 | 0·6 | 1·9 | — | — | — | — | — | 157 | 178 |
| 2 | 4·6 | 21·8 | 9·2 | 10·3 | 8·8 | 8·1 | 6·5 | 8·1 | 8·4 | 5·4 | 3·1 | 1·9 | 2·3 | 0·4 | 0·8 | 0 | 0 | 0·4 | — | — | — | 261 | |
| 3 | 3·4 | 8·1 | 8·4 | 11·0 | 12·6 | 11·0 | 14·8 | 8·4 | 7·2 | 5·1 | 3·0 | 3·0 | 2·5 | 0·8 | 0·4 | 0·4 | — | — | — | — | — | 237 | |
| 4 | 0·6 | 2·5 | 8·6 | 11·0 | 12·3 | 15·3 | 15·3 | 9·8 | 11·0 | 5·5 | 3·7 | 2·4 | 0 | 0 | 0·6 | 0·6 | 0·6 | — | — | — | — | 163 | |
| 5 | 0·4 | 5·1 | 10·2 | 9·7 | 10·2 | 10·7 | 13·9 | 13·4 | 12·2 | 5·1 | 3·2 | 2·3 | 1·3 | 1·3 | 0·9 | — | — | — | — | — | — | 216 | |
| 6 | 1·1 | 4·1 | 6·2 | 12·3 | 15·4 | 16·4 | 13·8 | 7·1 | 10·2 | 3·6 | 3·0 | 1·5 | 2·0 | 0 | 2·0 | 1·1 | — | — | — | — | — | 195 | |
| 7 | 1·2 | 5·6 | 9·2 | 11·2 | 13·2 | 15·2 | 13·6 | 8·0 | 8·0 | 6·8 | 4·4 | 1·6 | 1·2 | 0·8 | — | — | — | — | — | — | — | 250 | |
| 1 | 0 | 6·1 | 12·1 | 7·9 | 16·8 | 14·0 | 12·6 | 9·3 | 9·8 | 3·7 | 3·3 | 1·4 | 0 | 1·9 | 0·5 | 0 | 0·5 | — | — | — | — | 214 | 226 |
| 2 | 1·4 | 5·1 | 12·1 | 17·8 | 15·4 | 15·0 | 9·3 | 6·5 | 6·1 | 5·1 | 1·4 | 2·8 | 0·9 | 0 | 0·5 | 0 | 0·5 | 0 | 0·5 | — | — | 214 | |
| 3 | 0 | 10·0 | 10·0 | 8·1 | 14·9 | 10·9 | 14·0 | 12·2 | 7·2 | 3·6 | 3·6 | 1·8 | 2·3 | 0 | 0 | 0 | 0 | 0·5 | 0·5 | 0·5 | — | 221 | |
| 4 | 0·4 | 4·8 | 6·1 | 13·7 | 13·3 | 14·9 | 15·3 | 10·1 | 7·3 | 4·8 | 2·8 | 3·2 | 1·6 | 0·8 | 0 | 0 | 0·4 | 0·4 | — | — | — | 248 | |
| 5 | 0 | 1·6 | 6·7 | 9·8 | 14·5 | 13·3 | 18·0 | 16·5 | 6·3 | 5·5 | 3·1 | 3·1 | 0·4 | 1·2 | — | — | — | — | — | — | — | 255 | |
| 6 | 0 | 4·5 | 9·1 | 12·5 | 12·5 | 13·2 | 16·0 | 9·4 | 9·8 | 5·6 | 3·1 | 2·4 | 1·0 | 0·3 | 0 | 0 | 0·3 | — | — | — | — | 287 | |
| 7 | 0 | 1·6 | 5·2 | 9·6 | 18·7 | 18·7 | 16·3 | 10·4 | 10·0 | 4·0 | 2·8 | 0·8 | 0·4 | 0·4 | 0·4 | 0 | 0·4 | 0·4 | 0·4 | — | — | 251 | |
| 1 | 0·4 | 5·4 | 8·0 | 13·4 | 20·1 | 11·1 | 14·3 | 9·4 | 4·9 | 3·6 | 3·6 | 1·8 | 1·3 | 0·4 | 0·9 | 0 | 0·4 | 0·4 | 0 | 0 | 0·4 | 224 | 255 |
| 2 | 5·1 | 21·5 | 13·1 | 8·8 | 8·8 | 13·9 | 9·5 | 7·3 | 6·2 | 2·2 | 1·1 | 1·1 | 0·4 | 1·1 | — | — | — | — | — | — | — | 274 | |
| 3 | 1·7 | 15·9 | 15·9 | 12·3 | 9·6 | 11·6 | 10·3 | 9·0 | 5·6 | 4·0 | 1·7 | 1·0 | 0·7 | 0·3 | 0 | 0 | 0 | 0 | 0 | 0·3 | — | 301 | |
| 4 | 3·9 | 15·8 | 8·4 | 10·5 | 13·0 | 14·7 | 10·1 | 10·5 | 6·3 | 2·5 | 2·1 | 0·4 | 1·4 | 0 | 0·4 | — | — | — | — | — | — | 285 | |
| 5 | 4·3 | 10·2 | 10·9 | 8·6 | 9·2 | 11·5 | 11·9 | 9·9 | 7·6 | 6·3 | 4·3 | 1·3 | 2·6 | 0·3 | 0 | 0·3 | 0·6 | — | — | — | — | 303 | |

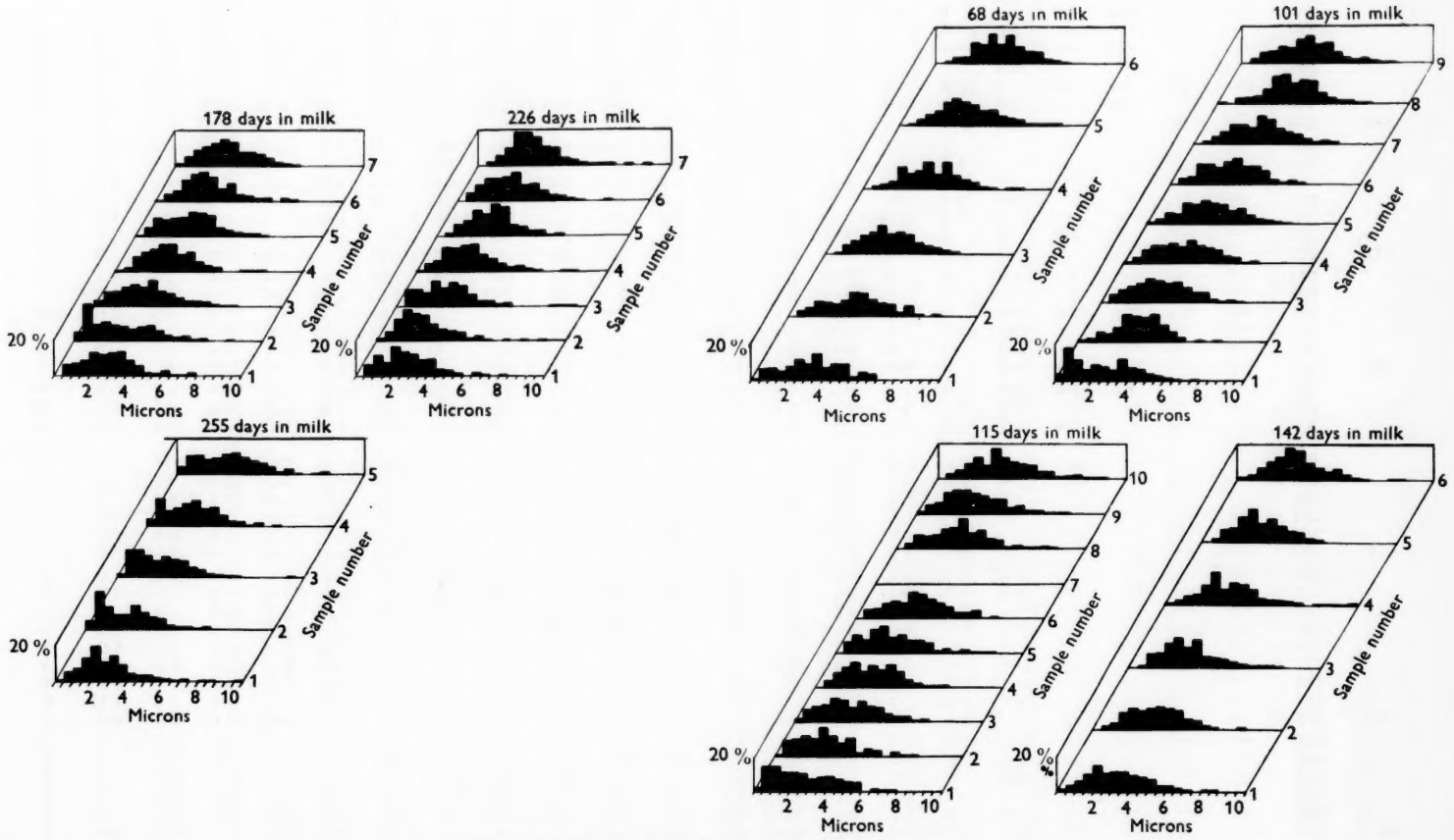



**Fig. 1. Fat-globule size distribution throughout lactation.**

Microns

Microns

Fig. 1. Fat-globule size distribution throughout lactation.

## DISCUSSION

*Trends between middle milk and strippings.* No obvious trends appear as the cow is being stripped or after Pitocin is injected. It is at this stage that the rapid increase in fat content occurs, so it is clear that whatever the cause of this fat increase, it is not associated with a marked change in fat-globule size.

*Trends between fore-milk and middle milk.* In a few cases the fore-milk samples contain more small globules than do later samples. It should be noted that there is difficulty in obtaining uniform fore-milk samples due to the unreliability of timing of let-down during the experiment. Sometimes the milk was down before the milking started; at other times, however, a few moments would elapse before let-down occurred. This influenced the nature of the milk sample collected and may explain why only a few samples show the predominance of small globules in the fore-milk. This predominance suggests that perhaps milk seeping into the large ducts and cistern prior to let-down does in fact undergo a limited filtration process, in which not only clusters but large globules are held back.

Johansson (6) has shown a noticeable trend in globule size between fore-milk and middle milk similar to that observed with some of our samples. He also shows a small change throughout the stripping phase. However, it does not seem likely that this small trend could explain the marked change in fat content at the end of milking. This point is discussed more fully in an earlier paper (4). Generally, Johansson's data are similar to those given here for the trends in globule distribution shown by milk during the stage in milking when the fat is rising quickly, and are probably best explained by the theory that clusters are filtered out by the small ductules and are ejected towards the end of milking.

*Change from p.m. to a.m. milking.* At 178 days in milk the samples which had previously been taken at the evening milking were taken in the morning. This continued throughout the rest of the experiment. The change appears to have had no effect on the size of the globules.

*Trends throughout lactation.* There is no evident trend with stage of lactation. The last set of samples shows a predominance of smaller globules, but without confirmation with more samples this may have no significance.

## GENERAL

The results confirm the earlier suggestion that the increase in the fat content of strippings cannot be explained as being due to the filtering out of large globules or creaming effects due to the rise of large globules. They also suggest that fore-milk may contain smaller globules than the rest, but this is indefinite because of the variability of the fore-milk sample. No distinct trends throughout milking or throughout lactation are to be seen.

## SUMMARY

An examination of the fat-globule size distribution pattern has been made throughout the lactation for one quarter of one cow, samples being taken at different stages in the milking process using a normal milking machine with sampling device attached.

The writer is indebted to Dr C. W. Turner and Mrs D. R. Perrin for the use of samples drawn in the course of another experiment.

## REFERENCES

(1) Whittlestone, W. G. (1952). *J. Dairy Res.* **19**, 127.
(2) Gaines, W. L. (1915). *Amer. J. Physiol.* **38**, 285.
(3) Petersen, W. E., Palmer, Z. S. & Eccles, C. H. (1929). *Amer. J. Physiol.* **90**, 592.
(4) Whittlestone, W. G. (1952). *Proc. N.Z. Dairy Sci. Ass.* 6.
(5) Eisenreich, L. & Mennicke, V. (1950). *Arb. Ber. Suddtsch. Vers- u. Forsch. Anst. Milchw. Weihenstephan*, **1**, 104.
(6) Johansson, I. (1952). *Acta agric. Scand.* **2**, 82.

(*MS. received for publication* 23 *April* 1953)

636.4:637.127

# 537. THE COMPOSITION OF SOW'S MILK DURING THE COURSE OF LACTATION

By DAWN R. PERRIN

*Ruakura Animal Research Station, New Zealand Department of Agriculture*

## INTRODUCTION

A knowledge of the chemical composition of sow's milk at various stages of lactation is of obvious importance in studies on the nutrition and management of both the sow and the litter. Furthermore, since the secretion and let-down of milk in the sow differ markedly from similar processes in the dairy cow, it is possible that a comparison of the chemical composition of the milk of the two species may help to elucidate some aspects of the general physiology of milk formation and secretion.

Few detailed analyses of sow's milk have been reported—probably because it is difficult to obtain adequate samples, as the sow gives her milk only while the pigs are suckling and so cannot be milked in the normal way. This difficulty in obtaining adequate samples has been mentioned in almost every report on the subject. Most workers have obtained only small samples while the sow was suckling her litter, a piglet being quietly removed and the vacated teat milked by hand.

In 1941 Ely & Petersen (1) induced let-down of milk in the dairy cow by intravenous injection of oxytocin. This method was applied by Braude, Coates, Henry, Kon, Rowland, Thompson & Walker (2) at Shinfield in 1947 to induce a similar milk let-down in the sow. After injection of oxytocin into the ear vein of the sow, the milk was withdrawn by hand from several teats. Several years later Bowland, Grummer, Phillips & Bohstedt (3) at Wisconsin extended this work, using an increased hormone injection and obtaining a larger sample. These methods certainly yielded an increased volume of milk, but with practical difficulties of hand milking by two workers, one on each side of the sow, and the limited time available in which to empty several teats, it is likely that the milk sample differed in volume and composition from the milk withdrawn by a litter during the natural suckling process. Bowland's efforts to withdraw milk samples by machine milking were unsatisfactory.

The development of a milking machine for sows (4) at this Station has made it comparatively easy to withdraw milk from a number of teats at the same time. The results obtained earlier indicate that the milk of different teats may vary in composition, especially in fat content (5). To obtain milk as close as possible in composition to that taken by the litter, it is necessary to withdraw from all the functional glands at the same time. The size of the samples taken for analysis has been regulated to that taken by the litter at an average suckling, in order not to upset the suckling habit and growth of the litter. It is considered that the samples withdrawn in this way are more representative of the milk drawn by the litter than were those available to earlier investigators. The analyses reported here were all made on samples of sow's milk obtained by this method, and have been carried out during studies on the nutritional requirements of the sow under different dietary levels, similar to those encountered in pig farming in New Zealand.

## EXPERIMENTAL

Analyses through sixty-eight lactations of forty-four sows are reported. The majority of the sows used were of the Berkshire breed, but some of the later lactations involved cross-bred Large White-Berkshire animals. The sows were milked at weekly intervals through the lactation period of each sow. Initially, the first sample was taken between the fourth and seventh days after farrowing, but as these early samples indicated that the composition changed rapidly over this period, especially with first-litter sows, only samples taken from the seventh day onwards are considered in this paper. The changes occurring in the first week after farrowing will be discussed in a later paper reporting studies on sow's colostrum. Variations in the ash constituents are not dealt with here, but are included in the subsequent paper as they are of especial interest in relation to the colostral stage.

On account of the wide variation in the weekly fat test of individual sows over the last lactation considered, the weekly analysis was done on a 3-day composite sample. The sows were milked, as already described, on three consecutive days and a composite sample prepared from portions from each milking. This was usually done on the basis of 10 ml. per ounce of milk yield, but in some lower producing sows it was necessary to use 15 or 20 ml. per ounce.

## ANALYTICAL METHODS

On account of the differences in composition between cow's and sow's milks, especially in the greatly increased protein and fat content of the latter and the associated difficulty in preparing a protein-free filtrate, the usual methods of milk analysis have had to be modified.

Fat content was determined by the Gerber Test (British Standards Institution, 1936) using an 8 ml. pipette for the milk, about 3 ml. of water being added after the addition of amyl alcohol. Initially on account of the smaller average size of the individual fat globules of sow's milk as compared with cow's milk, a comparison was made with Röse-Gottlieb procedure. It was found that the Gerber test is suitable as a rapid fat test for normal samples of sow's milk.

Total solids were determined by drying at 98–100° C. (British Standards Institution, 1951), the weight of milk sample being selected to give a dry residue weighing 0·6–0·8 g. Constant weight was usually attained after 8 to 10 hr. heating.

*Protein* (N × 6·38). About 2 g. milk was accurately weighed and diluted to 50 ml. with water. A 5 ml. aliquot was digested until clear with 1·8 ml. concentrated sulphuric acid to which was added 0·8 g. catalyst mixture (12 g. Se to 500 g. $K_2SO_4$). After cooling, the sides of the Kjeldahl flask were rinsed down with the minimum amount of hot distilled water and the flask contents were redigested for 30 min. The digest was diluted with about 10 ml. water, and the ammonia from the whole digest liberated in Parnas-Wagner steam distillation apparatus according to the method described by White Thompson & Brice (6).

*Lactose.* Over the course of this work, two different methods were used to estimate the lactose content. Initially when the number of samples was small and the size of the sample rather limited, a modification of the Luff-Schoorl copper reduction method for aqueous sugar solutions was used (Method I). Later when the number of samples per week increased substantially, this method was found to be very tedious, and as a larger

amount of each sample was available for chemical analysis, a change was made to a polarimetric procedure (Method II). Good agreement was found between these two procedures.

*Method I.* About 4 g. milk accurately weighed was diluted with a little water in a 100 ml. volumetric flask and deproteinized, using the minimum amount of tungstic acid (formed by the interaction in the solution of equivalent amounts of 10% sodium tungstate and $\frac{2}{3}$ N-sulphuric acid) to produce a clear filtrate. Following filtration, 25 ml. of the milk serum (containing about 50 mg. lactose) was boiled with 25 ml. copper citrate reagent according to the modified Luff-Schoorl method (7), the residual copper being estimated by liberation of iodine from potassium iodide and titration with sodium thiosulphate.

*Method II.* 50 g. of sow's milk was weighed into a 200 ml. volumetric flask and diluted with about 100 ml. water. Then 15 ml. acid mercuric nitrate solution followed by 15 ml. 5% phosphotungstic acid were added with gentle shaking. The flask was filled to the mark with water and allowed to stand for 15 min. during which time the stoppered flask was gently inverted on several occasions. The solution was filtered through a dry fluted paper, and the rotation of the filtrate was measured in a 4 dm. tube using the $Na_D$ line. The temperature correction suggested by Bacharach (8) was applied. To correct for the volume of what appeared to be a very large precipitate, some preliminary work was undertaken in which varying amounts of standard lactose solution were added to milk in flasks before the precipitation to produce a protein-free serum. Consistent results were obtained and as a result the value 10 ml. for the volume of the precipitate was used.

Ash was determined by drying 10 g. samples in an oven at 98–100° C. overnight and then incinerating in a muffle furnace below 550° C. to constant weight, 6–7 hr. being usually required.

## RESULTS AND DISCUSSION

### *Trends in composition throughout lactation*

The composition of the milk through sixty-eight lactations is shown in Table 1. The figures given are the mean values for all samples analysed at each period in lactation. The standard deviations from these mean values are also listed. The following trends are apparent from this table.

Table 1. *Mean values with standard deviations for constituents of sow's milk*

(Expressed as percentage by weight.)

| Days in milk ... | 7–13 | 14–20 | 21–27 | 28–34 | 35–41 | 42–48 | 49–56 |
|---|---|---|---|---|---|---|---|
| Total solids | 21·20±1·53 | 21·29±1·28 | 21·67±1·65 | 21·37±1·32 | 21·26±1·46 | 21·05±1·31 | 21·07±1·30 |
| Fat | 10·12±1·59 | 10·34±1·17 | 10·45±1·54 | 9·86±1·39 | 9·37±1·55 | 8·70±1·29 | 8·21±1·37 |
| Solids-not-fat | 11·08±0·37 | 10·95±0·39 | 11·22±0·42 | 11·51±0·34 | 11·89±0·43 | 12·35±0·48 | 12·86±0·52 |
| Protein | 5·35±0·78 | 5·31±0·44 | 5·60±0·53 | 5·92±0·54 | 6·35±0·58 | 6·84±0·43 | 7·38±0·71 |
| Lactose | 4·85±0·32 | 4·82±0·37 | 4·70±0·46 | 4·64±0·44 | 4·57±0·46 | 4·44±0·37 | 4·31±0·43 |
| Ash | 0·79±0·04 | 0·79±0·04 | 0·84±0·04 | 0·89±0·05 | 0·97±0·06 | 1·04±0·05 | 1·10±0·06 |

(*a*) *Fat.* The fat content shows a rise over the first 3 weeks followed by a fall over the remainder of the lactation.

(*b*) *Protein.* As will be apparent from results to be published in a later paper the protein content of sow's colostrum at parturition is high, generally more than 17%. The rapid fall in milk protein over the first week continues more slowly until a minimum is reached

about the sixteenth day after parturition. Thereafter it rises steadily to over 7% at the end of the lactation.

(*c*) *Lactose.* The lactose content remains relatively constant for over a fortnight, and then falls off slightly over the remainder of the lactation.

(*d*) *Total solids.* The total solids also show a rise over the first 3 weeks followed by a slight decline as lactation proceeds. This much less marked decline in the case of total solids is due to the opposing effects of an appreciably decreasing fat content and a slowly increasing solids-not-fat value.

(*e*) *Ash.* The ash content shows a general rise throughout the lactation, except between the second and third weeks, when it remains steady or may show a slight decline.

For almost all constituents the third week of lactation seems a critical stage.

Several workers have previously reported studies on lactational changes in the composition of sow's milk.

Willett & Maruyama (9) compared the fat contents of milk during early and advanced stages in lactation from sows fed on three different rations containing varying levels of garbage. They observed that the fat content of the milk increased with increase of fat intake and also with advance of lactation. They noted a greater variation in fat tests with the sows receiving the highest garbage ration, where the fat content was highest but not at a constant daily level.

The first detailed study of actual lactational trends in the different milk constituents was undertaken by Braude *et al.* (2). The milk was obtained at weekly intervals by manual expression as described earlier. They reported that after the rapid change from colostrum to milk, the fat content decreased appreciably and the solids-not-fat content increased slightly as lactation advanced. As a result of these opposing trends the total solids content showed a very slight tendency to decrease, but significantly so only in summer lactations.

Bowland *et al.* (3) made a more extensive study on similar lines, the technique of milk let-down and collection being similar to that of Braude. The sows were milked at parturition, the third day, the end of the first week and each subsequent week throughout the 8-week lactation period. They also observed that after an initial rise the fat content decreased appreciably as lactation advanced. They considered that it was difficult to establish a trend for total solids, but that a low point appeared to be reached about the third week of lactation after which the total solids increased slightly. In the case of solids-not-fat there was a tendency to rise after the third week. Protein showed a decline until the third week, after which it gradually rose. After an initial rise from the colostral stage, the lactose was more or less constant over the whole lactation. The ash showed a very definite rise from a little over 0·7% to between 1·2 and 1·3% in the eighth week milk.

In comparing the results obtained at this Station with those of other workers who have also studied lactational trends, the greatest difference is apparent in the fat figures. Willett & Maruyama (9) reported a definite increase with advancing lactation, whereas the results of Braude *et al.*, Bowland *et al.* and the present study all show an initial increase followed by an appreciable decrease as lactation advances. This trend is opposite to that in the dairy cow, and may be the result of some difference in the physiology of fat secretion between the two species.

However, in comparing the milk of the sow with the milk of other mammals, it must be emphasized that in the case of the sow the change in composition of sow's milk has

been observed only during the course of a lactation period of 56 days, at the end of which, when milking suddenly ceases, in many sows the milk production is still quite high.

The average fat content of the milk obtained at this Station is appreciably higher than the mean values reported by all other workers.

Trends in the other major constituents are similar to those observed by both Braude and Bowland and their associates. The protein content decreases until about the middle of the third week, and then rises steadily as lactation proceeds. The lactose remains steady until about this same stage, and then falls slightly over the rest of the lactation. Apart from a stationary stage during the third week, the ash content rises steadily over the whole lactation. The third week seems to be a critical time for several of the major constituents, because during this week there is a change in the direction of their trends. This does not coincide with the stage of maximum production, but appears some one or two weeks earlier.

Braude *et al.* found that the total solids content showed a very slight tendency to decrease. Bowland *et al.* reported a difficulty in establishing a trend for the total solids, but suggested that after a low point during the third week, there was a slight increase. The present study shows a rise until the fourth week, followed by a gradual decrease over the rest of the lactation. This difference is probably accounted for by the much higher fat content of the milk of the Ruakura sows compared with the animals used in the experiments at Shinfield and Wisconsin. Not only does the fat content attain a higher average value at the time when the total solids content is highest, but the change in fat content between early and late lactation is more marked. Since the changes in the other major constituents are smaller than the changes in the fat, the total solids content fairly closely follows the variation in fat value over the lactation.

It has been customary for previous workers on this subject to present mean values for the major constituents of the milk based on the range of samples they have analysed. For what they are worth, the mean values obtained from more than 450 analyses over the sixty-eight lactations reported in this paper are: fat 9·58%, protein 6·11%, lactose 4·62%, ash 0·92%. These mean values may be compared with those of other workers listed in Table 2. Only those derived from a reasonable number of samples from one or more groups of sows are included. Early results of a few analyses from an individual sow have not been listed.

Table 2. *Comparative analyses of sow's milk*

(Content of major constituents expressed as percentage by weight.)

| No. of sows | No. of samples | Total solids (%) | Fat (%) | Solids-not-fat (%) | Protein (%) | Lactose (%) | Ash (%) | Authority |
|---|---|---|---|---|---|---|---|---|
| 4 | 7 | 19·08 | 7·06 | 12·02 | 6·20 | 4·75 | 1·07 | Henry & Woll (1897) |
| 5 | 5 | 17·72 | 5·97 | 11·75 | 5·12 | 5·81 | 0·82 | Woll (1899) |
| 12 | 24 | 19·49 | 6·89 | 12·60 | 6·06 | 5·64 | 0·98 | Carlyle (1903) |
| 2 | 10–20 | 17·98 | 6·77 | 11·68 | 6·22 | 4·77* | 0·97 | Hughes & Hart (1934) |
| 6 | 12 | 16·96 | 5·99 | 10·97 | 5·07 | 5·09* | 0·81 | Vinogradsky (1939) |
| 35 | 43 | 16·35 | 5·29 | 11·06 | 4·86 | 5·28 | 0·92 | Wells *et al.* (1939) |
| 9 | 40–76 | 19·87 | 8·17 | 11·67 | 5·79 | 4·81 | 0·94 | Braude *et al.* (1947) |
| 20 | 32–67 | 20·08 | 6·81 | 13·27 | 7·26 | 5·12 | 0·99 | Bowland *et al.* (1949) |
| 44 | >450 | 21·23 | 9·58 | 11·65 | 6·11 | 4·62 | 0·92 | Present work |

* Calculated by difference.

Hughes & Hart (13) made an extensive review of the available literature on the gross

composition of sow's milk and calculated mean values for the combined results of other workers: total solids 18·18%, fat 6·85%, protein 6·19%, lactose 5·00% and ash 0·98%. Their own results based on two sows milked at 10-day intervals over the lactation gave results quite close to these mean values. The mean values of all results in the present study are similar to those reported by other workers. It must be emphasized, however, that the quite marked variations due to the stage of lactation make such average values meaningless for most practical purposes in studies on the nutrition of the lactating sow and her litter.

*Fluctuations in composition from day to day*

Under all the dietary levels studied at this Station the fat content of the milk of the sow varies considerably from day to day. This is illustrated by Table 3, in which analyses of the milk of two sows under study are listed for three consecutive days of each week.

Similar day-to-day variations in fat content have been followed through more than thirty lactations. The non-fat constituents also show some variation, but it is much less

Table 3. *Day-to-day variation in the major constituents*

(Expressed as percentage by weight.)

| Sow | Week | Yield (oz.) | Fat (%) | | Protein (%) | | Lactose (%) | | Ash (%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| X | 2nd | 8¼ | 11·10 | | 6·10 | | 4·13 | | 0·80 | |
| | | 6 | 9·30 | 9·60 | 5·49 | 5·57 | 4·55 | 4·46 | 0·78 | 0·77 |
| | | 6 | 8·40 | | 5·11 | | 4·70 | | 0·74 | |
| | 3rd | 5¾ | 10·20 | | 6·14 | | 4·55 | | 0·86 | |
| | | 9 | 6·70 | 9·23 | 6·31 | 6·00 | 4·03 | 4·49 | 0·83 | 0·85 |
| | | 4¾ | 10·80 | | 5·56 | | 4·88 | | 0·86 | |
| | 5th | 9½ | 8·30 | 8·23 | 6·67 | 6·86 | 4·28 | 4·04 | 0·97 | 0·97 |
| | | 8½ | 8·15 | | 7·05 | | 3·80 | | 0·97 | |
| | 7th | 8¼ | 6·95 | | 7·94 | | 4·01 | | 1·13 | |
| | | 10½ | 7·00 | 7·32 | 7·70 | 7·93 | 3·94 | 3·87 | 1·13 | 1·13 |
| | | 8 | 8·30 | | 8·14 | | 3·65 | | 1·13 | |
| | 8th | 9¾ | 6·70 | | 8·23 | | 3·96 | | 1·15 | |
| | | 7 | 6·75 | 6·88 | 8·35 | 8·56 | 4·29 | 4·04 | 1·16 | 1·18 |
| | | 9 | 7·20 | | 9·11 | | 3·87 | | 1·23 | |
| Y | 2nd | 7½ | 11·20 | | 5·37 | | 4·49 | | 0·76 | |
| | | 7½ | 9·85 | 11·25 | 5·25 | 5·28 | 4·53 | 4·45 | 0·78 | 0·78 |
| | | 7½ | 12·70 | | 5·23 | | 4·32 | | 0·81 | |
| | 3rd | 9½ | 11·25 | | 5·32 | | 4·34 | | 0·84 | |
| | | 10 | 10·70 | 11·00 | 5·83 | 5·60 | 4·42 | 4·20 | 0·88 | 0·87 |
| | | 9¾ | 10·95 | | 5·65 | | 4·12 | | 0·88 | |
| | 5th | 7 | 10·10 | 10·10 | 6·33 | 6·37 | 4·55 | 4·36 | 1·02 | 1·03 |
| | | 9 | 10·10 | | 6·40 | | 4·17 | | 1·03 | |
| | 7th | 9½ | 9·60 | | 7·25 | | 3·94 | | 1·13 | |
| | | 6 | 7·80 | 9·13 | 6·65 | 6·94 | 4·44 | 4·12 | 1·13 | 1·13 |
| | | 11¼ | 10·00 | | 6·91 | | 3·98 | | 1·12 | |
| | 8th | 10½ | 8·30 | | 7·02 | | 3·97 | | 1·17 | |
| | | 11 | 7·05 | 7·47 | 6·92 | 7·21 | 4·15 | 4·16 | 1·19 | 1·20 |
| | | 10½ | 7·05 | | 7·68 | | 4·37 | | 1·24 | |

marked than in the case of the fat. All these fluctuations seem to be greater in the early part of the lactation than later on. Although the individual values seem rather erratic, in general the average values support the trends mentioned earlier.

By preparing a composite sample for each sow from several milkings within the week, more representative samples have been obtained. Thus errors due to day-to-day variations in composition have been considerably reduced, and this procedure is being adopted for future studies at this Station.

*Variations in composition during milking*

Some preliminary work has been done on the fat content of sequential samples taken during a milking. It should be noted that our experience is that the litter at a normal suckling appears to withdraw about one-third to one-half of the milk actually present in the gland. Three or four successive injections, each of 3 units of Pitocin, were used to make the sow allow the withdrawal of all her milk, while sequential samples were taken during the milking. Some of these results are shown in Table 4.

Table 4. *Variation in fat content during milking*

| Sow | Days in milk | Fat (%) Sequential samples 1 | 2 | 3 | 4 | 5 | Amount taken during sampling (oz.) | Average amount taken by litter (oz.) |
|---|---|---|---|---|---|---|---|---|
| A | 11 | 8·60 | 7·75 | 10·55 | 9·55 | — | 13½ | 6 |
| | 39 | 7·40 | 7·45 | 7·60 | 7·70 | 7·95 | 14 | 7 |
| B | 23 | 13·35 | 12·70 | 13·80 | 11·95 | — | 24 | 11 |
| | 50 | 7·80 | 7·80 | 8·30 | 8·25 | 7·45 | 11½ | 11 |
| C | 14 | 7·25 | 7·65 | 9·00 | 9·40 | — | 6¾ | 3 |
| D | 56 | 7·35 | 7·35 | 7·45 | 7·50 | 7·45 | — | 6 |

There is no regular variation in fat content during milking as in the case of the cow. Sequential samples taken during the first part of the lactation show an erratic fat content, but towards the end of the lactation, the fat content appears less variable, sometimes remaining almost constant or else showing a slight rise as milking proceeds.

Except for results obtained at this Station (5, 16) no reference has been found to studies on the changes in composition of sow's milk during the process of milking. In the case of other workers where samples were obtained by hand milking and when time necessarily limited the amount of milk available, several recommendations have been made as to the advantage of completely milking-out several glands rather than withdrawing a smaller amount from a larger number of functional glands. This has probably been assumed on analogy with cow's milk when large variations are observed between the fat contents of the first- and last-drawn milk. There is no similar change in the fat content of sow's milk during the course of a milking, and as a result there is no basis for such a recommendation.

Variations in the solids-not-fat constituents have been studied on only one sow late in the lactation. When calculated on a fat-free basis, the protein, lactose and ash contents were found to be unchanged during the process of milking.

## SUMMARY

1. The composition has been studied of the milk of forty-four sows in the course of sixty-eight lactations. The milk was obtained by machine milking following intravenous injection of oxytocin.

2. Definite lactational trends have been observed for all the major constituents, and the third week seems a critical stage for most of the milk components.

3. The mean values for the milk, based on the analyses of over 450 samples from the seventh to fifty-sixth day of the lactation were: fat 9·58%, protein 6·11%, lactose 4·62%, ash 0·92%. Such figures are considered to be rather meaningless for most practical purposes, in view of the marked changes over the course of lactation.

4. The day-to-day variation in the composition of sow's milk has been studied. Whereas the fat value changes considerably, the non-fat constituents show a less marked variation. Difficulties in securing a representative sample of the weekly milk have been overcome by preparing a composite sample from three milkings within the week.

5. Some preliminary work has been done on variation in the composition during the process of milking. Sequential samples show an erratic fat content, especially during the early part of the lactation. When calculated on a fat-free basis, the protein, lactose and ash contents are relatively constant during the process of milking.

## REFERENCES

(1) Ely, F. & Petersen, W. E. (1941). *J. Dairy Sci.* **24**, 211.
(2) Braude, R., Coates, M. E., Henry, K. M., Kon, S. K., Rowland, S. L., Thompson, S. Y. & Walker, D. M. (1947). *Brit. J. Nutr.* **1**, 64.
(3) Bowland, J. P., Grummer, R. H., Phillips, P. H. & Bohstedt, G. (1949). *J. Dairy Sci.* **32**, 22.
(4) Smith, D. M., Whittlestone, W. G. & Allen, J. (1951). *J. Dairy Res.* **18**, 31.
(5) Smith, D. M. (1952). *Proc. N.Z. Soc. Anim. Prod.* **12**, 102.
(6) White, E. P., Thompson, F. B. & Brice, N. (1948). *Analyst*, **73**, 146.
(7) Browne, G. A. & Zerban, F. W. (1941). *Physical and Chemical Methods of Sugar Analysis*, 3rd ed. New York: John Wiley and Sons.
(8) Bacharach, A. L. (1923). *Analyst*, **48**, 521.
(9) Willett, E. L. & Maruyama, C. (1946). *J. Anim. Sci.* **5**, 365.
(10) Henry, W. A. & Woll, F. W. (1897). *14th Ann. Rep. Wis. agric. Exp. Sta.* p. 10.
(11) Woll, F. W. (1899). *Rep. Wis. agric. Exp. Sta.* no. 267.
(12) Carlyle, W. L. (1903). *Bull. Wis. agric. Exp. Sta.* no. 104, p. 8.
(13) Hughes, E. H. & Hart, H. G. (1935). *J. Nutr.* **9**, 311.
(14) Vinogradsky, A. I. (1939). *People's Commissariat of Grain and Animal State Farms of U.S.S.R.* **14**, 86.
(15) Wells, W., Beeson, W. M. & Brady, D. E. (1939). *Bull. Idaho agric. Exp. Sta.* no. 236.
(16) Whittlestone, W. G. (1952). *J. Dairy Res.* **19**, 127.

(*MS. received for publication* 23 *April* 1953)

636.4:637.127

# 538. THE ESTIMATION OF THE ENERGY OF SOW'S MILK FROM FAT AND TOTAL SOLIDS CONTENT

By DAWN R. PERRIN
*Ruakura Animal Research Station, New Zealand Department of Agriculture*

In studies on the efficiency of sow-milk production and litter growth, comparisons between individuals and groups of animals can best be made on the basis of energy production. Neither total milk production nor composition studies alone can provide sufficient information upon which to base conclusions. The energy value of the milk may be obtained either by direct calorimetry or, provided the conversion factor for each milk constituent is known, by calculation from the chemical composition of the milk. In the absence of a suitable calorimeter and because information on the chemical composition was also required, the latter method has recently been used at this Station.

## CONVERSION FACTORS FOR CALCULATION OF ENERGY

In order to calculate energy from the chemical composition of sow's milk calorific values obtained for the constituents of cow's milk have been used, since corresponding data for sow's milk, or indeed for milk of other mammals, are not available. However, as the calorific value of goat's milk can be estimated with considerable accuracy in this way from the fat, lactose and protein percentages (1), and the calorific value of human milk (2) agrees with the values expected from the chemical composition, it seems likely that the conversion factors are similar for different species. The only report of direct calorific determinations on sow's milk of known compositions is that of Vinogradsky (3), but no lactose contents are listed. Using his results, and estimating the percentages of lactose by difference, it is possible to calculate energy values from the composition which for most of the ten samples listed are in satisfactory agreement with values obtained by calorimetry.

Various combustion factors per gram of constituent have been reported for cow's milk. The standard values reported by Abderhalden (4) in 1908 and obtained directly for each constituent are: fat 9·23, protein 5·71 and lactose 3·95 Cal./g. These agree closely with those obtained in 1926 by Andersen (5), whose values are fat 9·11, protein 5·86 and lactose 3·95.

Overmann & Gaines (6) compared the direct-energy determination and the energy of the milk calculated from the chemical composition of 212 samples and obtained the values fat 9·25, protein 5·85 and lactose 3·69 Cal./g. These indirectly determined values agree with the standard values in the case of fat and protein, but much less closely for lactose. In this series of samples, lactose was determined by difference. It is possible that the conditions of drying used in determining the total solids of the milk may have left part or all of the lactose in the hydrated form, $C_{12}H_{22}O_{11}.H_2O$, in the residue. If allowance is made for complete hydration Gaines's factor for lactose becomes 3·88.

Overmann & Gaines found that the values for fat, protein and lactose fluctuated to some extent according to the components considered in calculating these values. Thus, using the same series of calorimetric and analytical results, if they also considered the

percentages of ash and water in the milk samples, they obtained a different set of conversion factors. If they considered fat, protein, lactose and ash they obtained a third set, differing slightly from the other two. This suggested that their method was not suited to the exact determination of the calorific value of milk components, but in attempting to make the most accurate estimate from their data they decided to consider all the milk components. Their justification for including water and ash was that there may be some peculiarity associated with high-water milk which would tend to make the energy determination slightly lower, and that similar reasoning might be applied to the ash. They decided that statistically the best energy estimate was obtained by use of all five components, that is

$$E = 9{\cdot}312\,F + 5{\cdot}358\,P + 3{\cdot}987\,L + 4{\cdot}980\,A - 0{\cdot}035\,W,$$

where $E$ is Cal./100 g. of milk and $F$, $P$, $L$, $A$ and $W$ are percentages of fat, protein, lactose, ash and water respectively.

In this present work, it is considered that an allowance for an energy contribution from the water or the ash content of the milk is unwarranted, and that rather than use these empirical values of Overmann & Gaines, it is preferable to use the directly determined factors of Andersen previously quoted.

Applying these conversion factors, the energy value of sow's milk can be calculated as follows:

$$E = 9{\cdot}11\,F + 5{\cdot}86\,P + 3{\cdot}95\,L. \qquad \text{(i)}$$

### CALCULATION FROM FAT AND TOTAL SOLIDS ONLY

The chemical composition studies at this Station(7) with forty-four different sows over sixty-eight individual lactations indicate that the ash and lactose contents of sow's milk show well-defined trends during the course of lactation and do not change significantly with varying dietary levels. These trends are shown in Table 1 (for thirty-four lactations). It appears possible therefore that for future work the determination of lactose can be avoided by using average values previously obtained. Similarly, an estimate of the protein content can be calculated from the solids-not-fat by subtracting values for lactose ($L'$) and ash ($A'$). The only determinations then required for assessing energy are fat and total solids.

Table 1. *Mean values for lactose and ash content of sow's milk*

| Time in lactation | $L'$ | $A'$ | $K'$ |
|---|---|---|---|
| 2nd week ( 7–13 days) | 4·88 | 0·79 | 13·95 |
| 3rd week (14–20 days) | 4·86 | 0·79 | 13·91 |
| 4th week (21–27 days) | 4·72 | 0·84 | 13·94 |
| 5th week (28–34 days) | 4·65 | 0·89 | 14·10 |
| 6th week (35–41 days) | 4·59 | 0·97 | 14·45 |
| 7th week (42–48 days) | 4·44 | 1·04 | 14·57 |
| 8th week (49–56 days) | 4·32 | 1·10 | 14·70 |

If the values $L'$ and $A'$ of Table 1 be accepted, the calculation for gross energy can be made from

$$E = 9{\cdot}11\,F + 5{\cdot}86\ (\text{S.N.F.} - L' - A') + 3{\cdot}95\,L'$$
$$= 9{\cdot}11\,F + 5{\cdot}86\ \text{S.N.F.} - K', \qquad \text{(ii)}$$

where

$$K' = (5{\cdot}86 - 3{\cdot}95)\,L' + 5{\cdot}86\,A',$$

and may be read, for the appropriate week, from Table 1.

COMPARISON OF STANDARD METHODS AND PROPOSED METHOD

Table 2 presents the calorific values of weekly samples of the milk of individual sows calculated in this way, from fat and total solids, $E_{(\text{calculated})}$ for comparison with the corresponding values calculated from the analyses for fat, protein and lactose, $E_{(F+P+L)}$.

Table 2

(Calories per 100 g. of milk.)

| | Week in lactation | | | | | | |
|---|---|---|---|---|---|---|---|
| | 2nd | 3rd | 4th | 5th | 6th | 7th | 8th |
| Sow I | | | | | | | |
| $E_{(F+P+L)}$ | 143·2 | 146·5 | 137·9 | 124·6 | 135·2 | 127·8 | 137·7 |
| $E_{(\text{calculated})}$ | 143·7 | 146·7 | 137·5 | 125·8 | 135·9 | 128·6 | 138·6 |
| Difference | +0·5 | +0·2 | −0·4 | +1·2 | +0·7 | +0·8 | +0·9 |
| Sow II | | | | | | | |
| $E_{(F+P+L)}$ | 133·7 | 139·9 | 139·1 | 142·9 | 132·3 | 134·1 | 148·7 |
| $E_{(\text{calculated})}$ | 133·9 | 140·4 | 139·6 | 142·8 | 132·6 | 134·5 | 149·1 |
| Difference | +0·2 | +0·5 | +0·5 | −0·1 | +0·3 | +0·4 | +0·4 |
| Sow III | | | | | | | |
| $E_{(F+P+L)}$ | 142·5 | 143·3 | 154·6 | 146·3 | 154·6 | 143·0 | 132·9 |
| $E_{(\text{calculated})}$ | 142·5 | 142·9 | 154·1 | 146·3 | 154·5 | 142·4 | 132·3 |
| Difference | Nil | −0·4 | −0·5 | Nil | −0·1 | −0·6 | −0·6 |
| Average | | | | | | | |
| $E_{(F+P+L)}$ | 139·8 | 143·2 | 143·9 | 137·9 | 140·7 | 135·0 | 139·8 |
| $E_{(\text{calculated})}$ | 140·0 | 143·3 | 143·7 | 138·3 | 141·0 | 135·2 | 140·0 |
| Difference | +0·2 | +0·1 | −0·2 | +0·4 | +0·3 | +0·2 | +0·2 |

Table 3. *Differences between average calorific values per* 100 *g. of milk obtained by standard and proposed methods*

$E_{(\text{calculated})} - E_{(F+P+L)}$

| | Week in lactation | | | | | | |
|---|---|---|---|---|---|---|---|
| Group | 2nd | 3rd | 4th | 5th | 6th | 7th | 8th |
| $A_1$ (4 sows) | +0·1 | +0·5 | +0·1 | +0·3 | −0·5 | +0·3 | +1·2 |
| $A_2$ (5 sows) | +0·5 | +0·2 | +0·1 | +1·0 | +0·9 | +0·1 | Nil |
| $B_1$ (5 sows) | +0·7 | +0·8 | +0·7 | +1·0 | +0·8 | +0·2 | −0·5 |
| $B_2$ (5 sows) | +0·8 | +0·7 | −0·3 | −0·3 | −0·7 | −0·1 | +0·3 |
| C (10 sows) | +1·0 | +1·1 | +2·0 | +0·4 | +0·4 | +0·1 | −0·6 |
| $D_1$ (5 sows) | −0·3 | +0·5 | +0·3 | +0·6 | +0·4 | +0·7 | +0·4 |
| $D_2$ (5 sows) | +0·4 | +0·1 | +0·5 | +1·0 | +0·4 | +0·3 | +0·3 |
| $D_3$ (4 sows) | −0·6 | +0·7 | +1·0 | +0·9 | +0·4 | −0·7 | +0·5 |
| $E_1$ (4 sows) | +0·7 | +0·9 | +1·0 | Nil | +0·4 | +0·4 | +0·1 |
| $E_2$ (5 sows) | +0·1 | +0·2 | +0·3 | +0·1 | +0·3 | +0·2 | +0·2 |
| $E_3$ (3 sows) | +0·2 | −0·2 | −0·2 | +0·4 | +0·3 | +0·2 | +0·2 |

N.B. Treatments C, D and E do not include any of the lactations used in derivation of the formula for $E_{(\text{calculated})}$.

Table 3 is based on results with groups of sows obtained at this Station over a period of several years and lists differences between the average calorific value for the group for each week in lactation calculated by both methods. The gross energy values all lie within the range 116–155 Cal.

Treatments A and B include some of the lactations from which the values of K′ in Table 1 were derived, but treatments C, D and E are entirely independent of the data from which the formula was devised.

### SOURCES OF ERROR

In most cases the difference between the two energy values is less than 1·0% of the gross energy of the milk. The greatest differences were observed in treatment C, the most extreme low dietary level yet tried at this Station. This series also exhibited the greater departures in composition from the average, when the lactose content was considerably lower and the protein content higher than had previously been observed for any group. In this series, the maximum difference between the group mean values of $E_{(\text{calculated})}$ and $E_{(F+P+L)}$ was 2·0 Cal., which is 1·2% of the energy value. The error introduced by variation of the lactose content under such extreme dietary treatment can also be illustrated from this series. Whereas the standard deviations from the mean lactose results listed in Table 1 are of the order of 0·4, in one individual sample in treatment C the lactose content fell 1·2 units below the average. The error in the calculation of $E$ resulting from this variation would still be less than 2% of the total energy.

It appears from the results in Tables 2 and 3 that the small differences between the gross energy values estimated by the two methods, that is $E_{(\text{calculated})} - E_{(F+P+L)}$, are more frequently positive than negative. Taking an average over the results listed, this difference is of the order of +0·2%. This amount is considered to be too small to warrant an additional factor to correct for it. It may be due to the non-ash component of the solids-not-fat being too high in the calculated series. Possibly the ash figure obtained by incineration is slightly lower than the true mineral content of the milk residue at the time of the total solids determination. A difference of only 0·07% in one of the constituents would account for the small positive bias of 0·2% in these results.

### SUMMARY

In studies where regular analyses of sow's milk are being made in an effort to evaluate the total energy content of the milk from sows at different dietary levels, once the composition trends of the milk have been assessed, the number of analyses can be considerably reduced and a greater number of samples handled. Satisfactory calorific values can be obtained from the estimated composition requiring analyses for fat and total solids only.

### REFERENCES

(1) Petersen, V. E. & Turner, C. W. (1939). *J. Nutr.* **17**, 293.
(2) Shukers, C. F., Macy, I. G., Nims, B., Donelson, E. & Hunscher, H. A. (1932). *J. Nutr.* **5**, 127.
(3) Vinogradsky, A. I. (1939). *People's Commissariat of Grain and Animal State Farms of U.S.S.R.* **14**, 86.
(4) Abderhalden, E. (1908). *Textbook of Physiological Chemistry in Thirty Lectures.* Transl. by W. T. Hall and G. G. Deffren. 722 pp. New York.
(5) Andersen, A. C. (1926). *Nord. JordbrForskn.* **4/7**, 133. (Beretn. nordisk. jordb. kongr. 3d. Oslo, Juni 1926.)
(6) Overmann, O. R. & Gaines, W. L. (1933). *J. Agric. Res.* **46**, 1109.
(7) Perrin, Dawn R. *J. Dairy Res.* **21**, 55.

(*MS. received for publication* 23 *April* 1953)

637.233.121

# 539. THE EFFECT OF TEMPERATURE TREATMENT OF CREAM BEFORE CHURNING ON THE CONSISTENCY OF BUTTER

By R. M. DOLBY

*The Dairy Research Institute (N.Z.), Palmerston North, New Zealand*

The consistency of butter depends to a large extent on the fatty-acid composition and glyceride structure of the butterfat present and is therefore affected by seasonal changes in the nature of the fat. In an earlier paper (7) it was shown that the hardness of New Zealand factory butters varies over a wide range from month to month through the dairying season. It was estimated that changes in composition of the butterfat were responsible for some 80% of these variations. This conclusion was in agreement with the finding from earlier experiments (6), viz. that moderate variations from the normal manufacturing conditions had very little effect on the hardness of the butter. It thus appeared that little could be done in commercial practice to offset seasonal fluctuations in butter consistency.

Workers in Europe, on the other hand, have claimed that it is possible, by suitable control of manufacturing conditions, to counteract the effect of seasonal variations in butterfat composition and thus to produce butter of a uniform consistency throughout the year. They have stressed the importance, for this purpose, of correct temperature treatment of cream before churning. Of the large number of papers on this subject we need quote here only sufficient to indicate the principles involved.

In 1937 Samuelsson & Pettersson (14) noted that cream cooled to 46° F. or lower for 2 hr. and then heated to 61° F. or higher yielded a butter which was softer and more greasy than that from cream which had not received a preliminary deep cooling. They found that 'temperature treatment' of cream, i.e. cooling followed by reheating, was an advantage in making winter butter (fat of low iodine value and high melting-point) but not with summer butter (fat of high iodine value and low melting-point).

Van Dam & Hovinga (16) confirmed this observation by showing that cream ripened at 66° F. after holding at 46° F. gave a softer butter than cream ripened at the same temperature without previous holding at a lower temperature. They also found that with cream held at 46° F. and subsequently ripened at different temperatures, the cream ripened at 54° F. gave firmer butter than that ripened at 66° F.

Storgårds (15) obtained softer butter from cream held at 57° F. than from cream held at 46° F. By centrifuging the butter granules from each churning, he was able to obtain samples of the free oil in the butter and found that the oil from the softer butter (cream held at 57° F.) had the higher iodine value.

Rapidly cooled fat was found by Adriani & Tamsma (1,2) (using a dilatometric method) to contain a maximum of crystalline fat at a given temperature, while fat which had been cooled in stages contained a minimum. On the basis of these findings they recommended that summer cream should be rapidly cooled to 57° F. and held at this temperature, while winter cream, after cooling to 57° F. and holding at this temperature for 8 hr., should be warmed to 72° F. for 1 hr., then cooled again to 57° F. and held at this temperature overnight.

Andersen (3) showed by similar methods that only 30% of the fat was crystallized in cream which had been held at 61° F. after souring at 66° F. If, however, the cream was first cooled to 46° F., before souring at 66° F. and holding at 61° F., 49% of the fat was in the solid state.

From a survey of the hardness, consistency grade and iodine values of 154 samples of Swedish commercial butter, Olsson (12) concluded that, while there is a certain correlation between iodine value of butterfat and hardness and consistency of butter, 'suitable treatment of the cream can, practically speaking, completely eliminate the impairing influence on the consistency of low or high iodine value'. Olsson indicated the following as correct 'temperature treatments' for creams containing butterfat of various iodine values:

| | Holding temperature (° F.) | | | |
|---|---|---|---|---|
| Iodine value | (1) | (2) | (3) | (4) |
| 27–31 | 46 | 66 | 63 | 55 |
| 31–34 | 46 | 66 | 59 | 55 |
| 34–37 | 48 | 64 | 52 | 52 |
| 37–43 | 48 | 59 | 50 | 50 |

(The temperatures indicated are, respectively, those: (1) immediately after cooling; (2) after 3 hr.; (3) in the evening; (4) the next morning.)

In a later paper (13) Olsson recommended that for summer butter (iodine value of fat 39–43) cream should be cooled after ripening instead of before ripening, suitable temperatures being 70–70–43–47° F. He also noted that summer cream cooled to a low temperature (41–46° F.) for 18–20 hr. gave butter with good consistency, but in this case it was necessary for the cream to be churned fresh or for the butter to be 'chemically ripened'.

Several attempts have been made to relate the effects of temperature treatment of cream and the consequent state of crystallization of the butterfat to the theory of butter structure developed by King. According to King & Fritz (9), in cream ready for churning the fat in each globule is partially crystallized in the form of a hollow shell of high melting fats enclosing a droplet of lower melting oil. During churning, oil is expressed from some globules while other globules are broken open, releasing the oil they contain. Churning is complete when sufficient oil has been released to cement the globules together to form butter granules. The finished butter consists of a dispersion of fat globules and water droplets in the 'free fat' which includes fragments of globules and crystalline non-globular fat together with the true continuous phase, liquid fat. During the first few days after churning, some of the liquid fat may crystallize, particularly if higher melting fats have been incorporated in it during churning and working.

King's theory has been amplified (e.g. Faxholm (8)) to explain differences in the body of butter. If the quantity of free liquid fat is more than sufficient to fill the spaces between the globules, the butter is soft and greasy. Where the proportion of oil in the fat is low, inclusion of some of the higher melting fraction of the fat with the free fat may take place during churning. This results in the presence of much crystalline fat in the free fat, and in such a case the butter will be hard and brittle.

The object of temperature treatment of cream before churning is to adjust the state of crystallization so that the correct amount of liquid fat is present in the globules. With fat of high iodine value (high oil content) the crystallization before churning should be as complete as possible, so that the amount of oil left free is only just sufficient to permit churning to take place, i.e. surplus oil in the finished butter should be retained in the fat

globules. With fat of low iodine value a smaller proportion of the fat should be crystallized and the crystallization should be arranged so as to give as complete a separation as possible of high and low melting fractions. The maximum amount of the oil fraction is thus allowed to escape into the free fat. Preliminary low cooling before holding at a temperature above churning temperature has been considered to assist in this respect.

In New Zealand butter-making practice, temperature treatment of cream during holding has differed widely from that adopted in European countries. Since a long-keeping butter is required, the acidity of the cream must be low and ripening with starter is omitted. The cream, after pasteurization, is cooled on direct expansion or plate coolers to approximately the temperature at which it is to be churned (40–50° F.) and is held at this temperature overnight. Previous investigations in New Zealand on the effect of temperature of holding cream before churning (6) were confined to a range of 5° F. above or below the churning temperature (in this case 40° F.). It seemed, therefore, that an investigation of the effect of a wider range of holding temperatures would be of interest.

## EXPERIMENTAL

### *Manufacture of butter*

Cream purchased from a commercial factory was bulked in a mixing vat, treated in a Vacreator, passed over two surface coolers (water and direct expansion), and was then divided between two or three water-jacketed direct-expansion holding vats. After being held for some 18 hr. at the appropriate temperatures, the batches of cream were churned in a small internal worker churn having a capacity of 150–200 lb. of butter. Parallel churnings were made under as nearly the same conditions as possible.

Samples of butter were stored at 40° F. for 5 or 6 days and then at 54·5° F. for 2 days before determinations of hardness were made. The bulk butter was stored at 14° F. for 4 months, after which it was again examined.

### *Experimental methods*

*Hardness* was determined by the sectility method (5) at 54·5° F. (12·5° C.). Results have been expressed as load in grams required to produce a rate of cutting of 0·01 cm./sec. ($F_{0·01}$).

*Free oil.* An indication of the quantity of 'free oil' (i.e. the liquid portion of the continuous phase) in the butter was obtained by determining the weight of oil exuding from slices of butter on to filter-paper at 64·4° F. (18° C.). This method has been used by Mohr & Baur (11) to indicate the 'oiling-off' tendency of butter at a temperature of 77 or 82·4° F. (25 or 28° C.). As it was desired in the present investigation to measure the free-oil content at a lower temperature, at which much less oil would exude, the following modification was adopted:

A plug of butter was taken with a large cork borer (diam. 7·25 cm.) fitted with a plunger actuated by a screw thread (16 threads per inch). Part of the plug was forced out by screwing down the plunger and the butter was trimmed off flush with the end of the borer by means of a wire. Successive slices, each $\frac{1}{16}$ in. (1·58 mm.) thick, were then cut off, the plunger being screwed down one turn before cutting each slice. A sheet of filter-paper (Whatman no. 2, 11 cm.) was pressed on to the exposed surface of the butter before each slice was cut, in order to make the slice easier to handle. Another sheet of filter-paper was placed on the other side of the slice of butter after it had been cut. A pile of four slices of butter was built up with two sheets of filter-paper on the top and bottom and

two sheets between each two slices of butter. Duplicate sets were prepared from each sample of butter, each set being separated from the next by a glass disk. The pile of samples was placed in a container in a water-bath maintained at 64·4° F. A 1 kg. weight was placed on top of the pile to keep the filter-paper and butter in good contact and the container was covered. The butter and filter-papers were removed 24 hr. later and were chilled for 30 min. in a refrigerator (as suggested by Mohr), after which the paper was peeled from the butter. The filter-papers from each set were weighed together and the increase in weight was noted.

The papers were then extracted with petroleum ether in a Soxhlet extractor. The ether was evaporated off on the water-bath and the last traces of solvent were removed from the oil by heating *in vacuo* for a few minutes. The softening point of the oil was determined by the method of Barnicoat (4) with a slight modification necessary on account of the low softening point: heating was commenced at 50° F. (10° C.) instead of 68° F (20° C.).

In the earlier experiments the weight of oil was determined from the increase in weight of the filter-paper after it had been left in contact with butter as described above (Method A). The extracted oil in this case was used only for the determination of the softening point. In one experiment on partly worked butter it was found that where the butter was 'leaky', the paper took up water as well as oil. An attempt was made to eliminate errors due to this cause by drying the filter-paper overnight in a vacuum desiccator before each weighing. (Oven drying was avoided on account of the likelihood of oxidation of the oil at high temperatures.) The results, however, were somewhat erratic because of difficulty in ensuring that the initial and final moisture contents of the paper were the same. It was found preferable, therefore, to determine the weight of oil directly by weighing after extraction (Method B).

This method of determination of the amount of free oil in butter is to be regarded as comparative. Only a part of the oil is removed, viz. that close to the butter surface in contact with the filter-paper. The results have therefore been reported as the weight of oil exuding from eight standard slices (45–50 g.) rather than as a percentage of the weight of butter taken. The amount and composition of the oil vary markedly with variation in the temperature at which the test is carried out. The results for batches tested at different times are not strictly comparable, but as the samples to be compared were always run through in the same batch, the conditions were the same for each sample in the batch. Duplicate determinations in the same batch showed good agreement, the average difference between duplicates being 0·1 g. on an oil weight of 1–2 g.

A more direct comparison of hardness and free-oil values would have been possible if these could have been measured at the same temperature. The hardness method, however, is unsuitable for use at temperatures where the butter is soft, while the amount of free oil which could be obtained at 60° F. or lower would be very small.

## Results

### (1) *Experiments on holding cream before churning at a temperature higher than that at which it was to be churned*

The effect of high holding temperature as used in European countries was studied by making parallel churnings of cream held at 60 and at 46° F., both lots of cream being churned at 45° F. Cream which had been held at 60° F. was cooled on a direct-expansion surface cooler immediately before churning.

The results of three replications of this experiment given in Table 1 show that the higher holding temperature caused a marked decrease in hardness of the butter and an equally pronounced increase in the free-oil content of the butter.

Table 1. *Effect of holding temperature of cream on hardness and free-oil content of butter*

Treatment: (1) Off cooler at 46° F., held at 46° F., churned at 45° F.
(2) Off cooler at 62° F., held at 60° F., churned at 45° F.

| Dates ... ... ... ... ... | 16. ix. | 23. ix. | 15. x. | Av. |
|---|---|---|---|---|
| Hardness (7 days): (1) | 340 | 300 | 320 | 320 |
| (2) | 220 | 170 | 230 | 210 |
| Free oil in butter (7 days) (Method A): (1) | 2·0 | 2·0 | 2·8 | 2·3 |
| (2) | 4·0 | 3·4 | 4·6 | 4·0 |
| Softening point of free oil (° F.): (1) | 63·4 | 64·0 | 61·4 | 62·9 |
| (2) | 65·6 | 64·6 | 60·6 | 63·6 |

The influence of slow cooling of cream to the churning temperature was investigated in a further series of experiments. The cream was cooled to 60° F. on the surface coolers, and its temperature while in the holding vat was reduced at such a rate that it required 12–15 hr. to reach the churning temperature (46° F.). Parallel churnings were made from cream held at 60° F. and at the churning temperature (46° F.). The results of these experiments are set out in Table 2.

Table 2. *Effect of rate of cooling and of holding temperature of cream on hardness and free-oil content of butter*

Treatment: (1) Off cooler at 48° F., held at 46° F., churned at 46° F.
(2) Off cooler at 60° F., cooled overnight to 46° F., churned at 46° F.
(3) Off cooler at 66° F., held at 60° F., churned at 46° F.

| Dates ... ... ... ... ... | 21. x. | 5. xi. | 26. xi. | 2. xii. | Av. |
|---|---|---|---|---|---|
| Hardness (7 days): (1) | 430 | 480 | 440 | 540 | 475 |
| (2) | 340 | 380 | 400 | 400 | 380 |
| (3) | 270 | 310 | 320 | 340 | 310 |
| Hardness (4 months): (1) | 340 | 480 | 480 | 540 | 460 |
| (2) | 320 | 420 | 430 | 450 | 405 |
| (3) | 300 | 370 | 390 | 400 | 365 |
| Free oil in butter (7 days) (Method A): (1) | 2·1 | 3·2 | 2·3 | 1·6 | 2·3 |
| (2) | 3·0 | 4·8 | 2·9 | 2·5 | 3·3 |
| (3) | 4·5 | 5·8 | 4·3 | 4·0 | 4·7 |
| Softening point of free oil (° F.): (1) | 61·2 | 63·8 | 62·4 | 64·4 | 63·0 |
| (2) | 61·4 | 63·4 | 62·6 | 62·6 | 62·5 |
| (3) | 60·6 | 61·2 | 62·4 | 61·6 | 61·4 |

The butter from cream held at 60° F. (treatment 3) was again found to be definitely softer and to contain more free oil than butter from cream held at the churning temperature (treatment 1). Butter from cream cooled slowly to churning temperature (treatment 2) was intermediate in hardness and free-oil content.

Trials were made of the 'temperature treatment' recommended by Olsson (12) for cream of the same iodine value as that available at the time at the Institute. The recommended procedure involved cooling the cream to 47° F., warming it in the vat to 61° F., cooling again to 54° F. and holding and churning at this temperature. The butter from cream treated in this way was similar in hardness and free-oil content to that from cream held overnight at 60° F. and churned at 47° F. The churning temperature of 54° F., however, proved to be too high for the type of cream being handled and resulted in high fat losses

in churning and a poor body in the butter. The following modified treatment was therefore adopted: Cream was taken off the surface cooler at 48° F., warmed in the vat to 60° F. and held at that temperature overnight. Immediately before churning it was cooled to 48° F. The results from parallel churnings of cream treated in this way and of cream held at 60° F. or at 48° F. are given in Table 3.

Table 3. *Effect of holding temperature of cream and of precooling before holding on hardness and free-oil content of butter*

Treatment: (1) Off cooler at 48° F., held at 48° F., churned at 48° F.
(2) Off cooler at 48° F., held at 60° F., churned at 48° F.
(3) Off cooler at 65° F., held at 60° F., churned at 48° F.

| Dates ... ... ... ... ... | 21. i. | 24. ii. | 10. iii. | 24. iii. | 31. iii. | Av. |
|---|---|---|---|---|---|---|
| Hardness (7 days): (1) | 420 | 450 | 420 | 390 | 420 | 420 |
| (2) | 280 | 330 | 270 | 280 | 260 | 285 |
| (3) | 310 | 320 | 300 | 320 | 300 | 310 |
| Hardness (4 months): (1) | 510 | 460 | 440 | 410 | 480 | 460 |
| (2) | 360 | 340 | 340 | 330 | 290 | 330 |
| (3) | 360 | 320 | 300 | 320 | 330 | 325 |
| Free oil in butter (7 days) (Method B): (1) | — | 1·6 | 2·0 | 1·7 | 2·0 | 1·8 |
| (2) | — | 3·0 | 2·1 | 3·3 | 4·1 | 3·1 |
| (3) | — | 3·8 | 2·6 | 3·6 | 4·2 | 3·6 |
| Free oil in butter (4 months) (Method B): (1) | 1·2 | 1·4 | 1·9 | 1·2 | 1·4 | 1·4 |
| (2) | 1·8 | 2·3 | 2·7 | 2·3 | 2·6 | 2·3 |
| (3) | 2·3 | 3·0 | 3·0 | 2·8 | 2·8 | 2·8 |
| Softening point of free oil (7 days) (° F.): (1) | 63·4 | 64·8 | 64·2 | 62·6 | 63·6 | 63·7 |
| (2) | 60·8 | 63·4 | 61·8 | 61·8 | 62·6 | 62·1 |
| (3) | 61·8 | 64·6 | 63·2 | 62·6 | 63·4 | 63·2 |
| Softening point of free oil (4 months) (° F.): | | | | | | |
| (1) | — | 63·0 | 61·8 | 63·4 | 61·8 | 62·5 |
| (2) | 61·2 | 61·2 | 60·4 | 60·8 | 59·5 | 60·6 |
| (3) | 62·0 | 61·6 | 61·2 | 61·4 | 60·6 | 61·4 |
| Fat in buttermilk: (1) | 1·3 | 1·4 | 1·6 | 1·7 | 1·8 | 1·56 |
| (2) | 1·3 | 1·6 | 1·8 | 1·8 | 2·0 | 1·70 |
| (3) | 2·0 | 1·7 | 2·3 | 2·5 | 3·0 | 2·30 |

Both treatments (nos. 2 and 3, Table 3) which involved holding cream at 60° F. resulted in butter which was softer and had higher free-oil content than butter from cream held at 48° F. Precooling to 48° F. before holding at 60° F. (treatment no. 2) yielded a butter which was slightly softer at 7 days than that from cream held at 60° F. without precooling. The difference in hardness, however, disappeared after storage.

The free-oil content of the butter was slightly lower where the cream was precooled (treatment no. 2) than where precooling was omitted (treatment no. 3), but the softening point of the oil was lower with no. 2 than no. 3. It appeared, therefore, that the precooling had resulted in a more complete separation of high and low melting-point constituents of the fat.

It was claimed by Olsson (12) that correct 'temperature treatment' of cream (i.e. precooling before holding) resulted in reduced losses of fat in the buttermilk. The results given at the foot of Table 3 support this claim. There were considerably higher fat losses with treatment no. 3 than with the control (no. 1), while treatment no. 2 gave a barely significant increase in losses over those in no. 1. The values for fat losses quoted should not be taken as indicative of those which would occur in commercial practice, since it has been noted that with the small experimental churnings (1½ cwt.) the fat losses are higher and more variable than those in commercial butter-making where churnings are

20–50 cwt. The experimental results can, however, be taken as showing the relative magnitudes of the losses.

A comparison of hardness and free-oil contents of the samples before and after cold storage shows that the respective orders of hardness and free-oil content had changed very little. In most cases the hardness had increased after storage, but the increase varied from sample to sample. There was also a decrease in the amount of free oil and in the softening point of the free oil. It seems likely that crystallization of the fat in the continuous phase was not quite complete when measurements were made at 7 days, but was possibly more complete in some samples than in others. The occasional softening point values over 64·4° F. are also probably due to a difference in the extent and type of crystallization of the fat in the butter and in the softening-point sample respectively.

### (2) *Experiments on holding cream before churning at a temperature lower than that at which it was to be churned*

The experiments described above have shown that it is possible to produce a considerable reduction in hardness of butter by raising the temperature at which cream is held before churning. It seemed possible that the converse relationship might apply and that the hardness of butter could be increased by a reduction in holding temperature of the cream. Such a treatment would be useful with spring butter, which in New Zealand is softer than butter made at other times of the year and which is frequently criticized by graders as being greasy and lacking in firmness. These experiments were commenced too late in the season for spring cream to be available, but the effect of low holding temperature was investigated and the experiments were continued in two following seasons during the spring period.

In Table 4 are given the results of a number of comparisons in which cream for one

Table 4. *Effect of low holding temperature of cream on hardness and free-oil content of butter*

Treatment: (1) Off cooler at 46° F., held at 46° F., churned at 46° F.
(2) Off cooler at 46° F., held at 35° F., churned at 46° F.

| Dates ... ... | 30. ix. | 7. x. | 14. x. | 11. xi. | 18. xi. | 14. i. | 3. ii. | 3. iii. | 16. iii. | Av. |
|---|---|---|---|---|---|---|---|---|---|---|
| Hardness (7 days): (1) | 320 | 380 | 340 | 480 | 450 | 410 | 460 | 440 | 400 | 410 |
| (2) | 330 | 420 | 350 | 450 | 450 | 400 | 490 | 440 | 420 | 415 |
| Hardness (4 months): | | | | | | | | | | |
| (1) | 290 | — | — | 360 | 350 | 470 | 430 | 470 | 450 | 400 |
| (2) | 280 | — | — | 330 | 350 | 450 | 460 | 470 | 470 | 400 |
| Free oil in butter (7 days) (Method A): | | | | | | | | | | |
| (1) | 2·3 | 2·6 | 2·8 | 2·1 | 2·3 | 3·4 | 6·1* | 1·1 | 1·2 | 2·7 |
| (2) | 2·2 | 2·3 | 3·1 | 2·4 | 2·4 | 3·4 | 5·5* | 1·2 | 1·0 | 2·5 |
| Free oil in butter (4 months) (Method B): | | | | | | | | | | |
| (1) | 2·8 | — | — | 2·4 | 2·6 | 1·3 | 1·6 | 1·9 | 1·1 | 2·0 |
| (2) | 3·1 | — | — | 2·2 | 2·3 | 1·2 | 1·7 | 1·5 | 1·0 | 1·9 |
| Softening point of free oil (7 days) (° F.): (1) | — | 59·8 | 61·4 | 62·4 | 62·6 | 62·6 | 68·7 | 64·4 | 62·8 | 63·1 |
| (2) | — | 62·0 | 61·7 | 62·0 | 62·8 | 63·2 | 67·1 | 65·4 | 63·7 | 63·5 |
| Softening point of free oil (4 months) (° F.): | | | | | | | | | | |
| (1) | 62·6 | — | — | 62·8 | 62·8 | 64·2 | 63·0 | 63·0 | 61·5 | 62·8 |
| (2) | 62·8 | — | — | 63·4 | 63·2 | 65·4 | 62·4 | 66·0 | 63·2 | 63·8 |

* Temperature exceeded 64·4° F.

churning was cooled to 46° F., held and churned at that temperature, while cream for the second churning was brought off the cooler at the same temperature, cooled in the vat as quickly as possible to 35° F., held at this temperature until shortly before churning and then warmed to 46° F.

It was found that a reduction in the temperature at which the cream was held before churning produced no significant change in the hardness of the butter. The change in quantity and softening point of the free oil was also insignificant.

The effect of a reduction in both holding and churning temperatures was then investigated. The results are set out in Table 5.

Table 5. *Effect of low holding and churning temperatures on hardness and free-oil content of butter*

Treatment: (1) Off cooler at 44° F., held at 40° F., churned at 41° F.
(2) Off cooler at 44° F., held at 34° F., churned at 35° F.

| Dates ... ... ... ... ... ... | 1. ix. | 16. ix. | 23. ix. | 30. ix. | Av. |
|---|---|---|---|---|---|
| Time to churn (min.): (1) | 23 | 20 | 27 | 37 | 29 |
| (2) | 33 | 65 | 54 | 70 | 56 |
| Hardness (7 days): (1) | 240 | 270 | 270 | 290 | 265 |
| (2) | 240 | 290 | 270 | 280 | 270 |
| Hardness (4 months): (1) | 230 | 300 | 290 | 350 | 290 |
| (2) | 250 | 340 | 260 | 310 | 290 |
| Free oil in butter (7 days) (Method B): (1) | 3·0 | 2·4 | 2·6 | 2·8 | 2·7 |
| (2) | 3·3 | 2·9 | 2·8 | 3·1 | 3·0 |
| Softening point of free oil (7 days) (° F.): (1) | 62·0 | 63·4 | 61·8 | 62·6 | 62·4 |
| (2) | 62·0 | 63·6 | 62·4 | 62·8 | 62·7 |

Even when holding and churning temperatures were both reduced there was no increase in hardness of the butter. The increase in churning time probably counteracted any effect of the lower holding and churning temperature, since where churning is prolonged there is a considerable rise in temperature before churning is completed. In one churning commenced at 35° F. the cream temperature had risen to 46° F. before churning took place.

Further experiments in the spring of the following season confirmed the conclusions that there was no advantage in reducing churning temperature to a point which gave a marked increase in churning time.

It was concluded that the method at present in commercial use in New Zealand gives the most satisfactory results for spring butter, and that no improvement in consistency of butter would result from the use of a holding temperature lower than the churning temperature. The cream, however, should not be held at a temperature above the churning temperature, as this would be likely to make the butter even softer.

## Discussion

It has been shown that if cream which is to be churned at 45–46° F. is held at 60° F. before churning instead of at the churning temperature, the resultant butter is considerably softer and contains more free oil. This observation is in accord with the experience of overseas workers, e.g. Storgårds (15). It is evident that, in cream held at 60° F. and cooled immediately before churning, the crystallization of the fat at the commencement of churning is less complete than it is in cream which has been held at the churning temperature. There is thus a better opportunity for oil to escape into the continuous phase of the

butter. The greater proportion of free oil accounts for the reduction in hardness of the butter.

If cream is cooled slowly (over some hours) to the churning temperature, the butter is softer and contains more free oil than butter from cream which has been cooled quickly to churning temperature and held at that temperature. This effect of rate of cooling of cream was observed in earlier experiments in New Zealand (6,17). It also can be attributed to the state of crystallization of the fat which was shown by Adriani & Tamsma (1), as quoted earlier, to be less complete in slowly cooled than in rapidly cooled cream. An additional effect of slow cooling is that the crystals formed will be larger and so will trap less fat than the fine crystals formed by more rapid cooling.

The butter from slowly cooled cream, although softer than butter from cream held at churning temperature, was not as soft as that from cream held at 60° F. and cooled immediately before churning. It would appear, then, that cream held at 60° F. contains less crystalline fat at churning than does cream treated by the other two methods mentioned.

The finding of European workers (14,16) that cream precooled to 46° F. before being held at 61° F. gave softer butter than cream held at the same temperature without precooling, has not been confirmed. In the present experiments, precooling of cream caused only a very slight reduction in hardness of butter, and even this difference disappeared after the butter had been stored. It is possible that differences in the methods used for the initial cooling of the cream may account for the discrepancy. The data on softening points of the free oil did, however, suggest that the precooling promoted a somewhat more complete separation of high and low melting constituents of the butterfat. The claim of Olsson (12) that precooling caused a reduction in fat losses in churning was also confirmed. Cream held at 60° F. without precooling gave considerably greater fat losses in churning than did cream held at 45° F. If the cream was precooled to 45° F. before being held at 60° F. the losses were little more than with cream held at 45° F. throughout the holding period.

An attempt to increase the firmness of butter, by holding cream at a temperature lower than that at which it was to be churned, proved unsuccessful. The reduction in holding temperature caused no significant increase in hardness or decrease in free-oil content of butter. It would appear that additional fat crystals, formed during the lower holding period, melted when the cream was warmed to churning temperature. Initial rapid cooling followed by holding at the churning temperature must, therefore, have produced the maximum crystallization possible at that temperature.

These results indicate that the firmness of butter containing fat of high iodine value cannot be increased by the use of lower temperatures during either part or the whole of the holding period. A lowering of both holding and churning temperatures together to the stage at which churning times were prolonged was also shown to be ineffective.

It may be concluded that the methods used in New Zealand tend to yield butter of almost the maximum hardness for the particular type of butterfat available at each stage of the season. This may be a partial explanation of the difference in hardness between New Zealand and European butters. (Lyons (10) found New Zealand butter to be considerably harder than Danish butter containing fat of similar melting-point and iodine value.)

In New Zealand commercial butter-making there is at present very little variation in the temperature treatment of cream before churning other than that which follows from

the necessary changes in churning temperature (from about 40° F. in spring when the fat is soft to 50° F. later in the season when it is hard). Although these changes in holding and churning temperatures will have some influence on the hardness of the butter, it is clear from results quoted earlier (7) that they do little to counteract the effect of seasonal variation in fat composition. Since no way has yet been found to increase the firmness of spring butter (high iodine value) beyond that obtained by present methods, uniformity could be achieved only by reducing the hardness of butter made at other times of the year. This could readily be done by use of higher holding temperatures before churning and would result in an improvement in spreadability with much of the butter which is now rather over-firm for use at winter temperatures. Such a change would be desirable in butter which is patted before export. At the present time, however, New Zealand butter is exported in bulk and is either blended or patted after arrival in England. Under these circumstances the firm body of New Zealand butter is considered an advantage, and the reworking involved in blending or patting causes an improvement in spreadability which, unless the butter is held too long after patting, should be maintained until the butter reaches the consumer.

SUMMARY

1. Measurements of hardness and 'free-oil' content of samples of butter from cream held at various temperatures before churning were made before and after storage. Hardness was determined by the sectility method, while free-oil content was estimated from the weight of oil exuding from thin slices of butter sandwiched between filter-papers and held at 64·4° F. (18° C.).

2. Butter from cream held at 60° F. and churned at 45° F. was much softer and contained more free oil than butter from cream held and churned at 45° F.

3. If the cream was precooled to 45° F. before being held at 60° F. the butter was very slightly softer than that from cream held at 60° F. without precooling, but the difference disappeared after storage. It contained rather less free oil, but the softening point of the oil was lower. Fat losses in churning which were high where cream was held at 60° F. were reduced almost to normal when cream was precooled before being held at 60° F.

4. Cream slowly cooled to 45° F. yielded butter which was softer and contained more free oil than that from cream cooled rapidly to 45° F. and held at that temperature. The butter, however, was not so soft as that from cream held at 60° F.

5. Butter from cream held at a temperature below that at which it was to be churned did not differ significantly in hardness or free-oil content from that produced from cream held at the churning temperature.

6. Present commercial methods of cream treatment in New Zealand (rapid cooling to churning temperature and holding at that temperature) give butter of almost maximum hardness for the type of fat involved at each stage of the season.

7. More uniform consistency of butter throughout the season could be achieved only by reducing the hardness of the firmer butter and not by increasing the firmness of the ofter butter.

The thanks of the author are due to Mr J. A. Singleton, Superintendent, and Mr J. J. O'Dea, buttermaker of the Dairy Research Institute (N.Z.) experimental factory, for arranging and carrying out the cream treatments, and to Dr F. H. McDowall for helpful advice and criticism.

## REFERENCES

(1) ADRIANI, W. & TAMSMA, A. F. (1941). *Versl. RijkslandbProefst., 's Grav.*, no. 47 (12), G, p. 941.
(2) ADRIANI, W. & TAMSMA, A. F. (1946). *Versl. RijkslandbProefst., 's Grav.*, no. 52 (1), G, p. 1.
(3) ANDERSEN, K. P. (1949). *Beretn. Forsøksm., Kbh.*, **63**, 38.
(4) BARNICOAT, C. R. (1944). *Analyst*, **69**, 176.
(5) DOLBY, R. M. (1941). *J. Dairy Res.* **12**, 329.
(6) DOLBY, R. M. (1941). *J. Dairy Res.* **12**, 344.
(7) DOLBY, R. M. (1949). *J. Dairy Res.* **16**, 336.
(8) FAXHOLM, H. (1946). *Nordisk. Mejeritidsskr.* **12**, no. 7.
(9) KING, N. & FRITZ, W. (1948). *Milchwissenschaft*, **3**, 2–12, 36–41, 75–82, 102–7.
(10) LYONS, J. (1936). *Econ. Proc. R. Dublin Soc.* **3**, 19.
(11) MOHR, W. & BAUR, K. (1948). *Milchwissenschaft*, **3**, 17.
(12) OLSSON, T. (1948). *Medd. Mejeriförs. Malmo*, no. 24, 1.
(13) OLSSON, T. (1950). *Medd. Mejeriförs. Malmo*, no. 29, 1.
(14) SAMUELSSON, E. & PETTERSSON, K. I. (1937). *Svenska Mejeritidningen*, **29**, 65, 73.
(15) STORGÅRDS, T. (1938). *Maataloust. Aikakausk*, **9**, 257.
(16) VAN DAM, W. & HOVINGA, G. (1938). *Versl. RijkslandbProefst., 's Grav.*, no. 44 (16), C, p. 739.
(17) VALENTINE, G. M. & SARGENT, J. D. (1935). *N.Z. J. Sci. Tech.* **16**, 206.

(*MS. received for publication* 2 *July* 1953)

637.232

# 540. A DEVICE FOR RAPIDLY CHURNING SMALL QUANTITIES OF CREAM

By R. M. DOLBY

*The Dairy Research Institute (N.Z.), Palmerston North, New Zealand*

(With 1 Plate and 1 Text-figure)

In studying the composition of butterfat from individual cows, the usual preliminary step in isolating the fat is to churn samples of cream separated from the milk. Conventional churns, such as the small glass household churns commonly used for this purpose, are inconvenient owing to the time taken to churn. It is, therefore, necessary to employ a battery of them to avoid delay when a number of samples have to be handled. Churns of this type are unsuitable also where the volume of cream available is much smaller than 500 ml.

In earlier work at this Institute batches of samples were churned in bottles mounted on an 'end-over-end' shaking machine. With small samples the thickened cream tended to stick to the bottles, with the result that the churning stopped at the 'whipped cream' stage and the process had to be completed by hand, e.g. by using a spatula in each bottle in turn.

Trials with a high-speed agitator in a covered beaker showed that complete and rapid churning was obtained, provided the beater swept the whole of the space enclosed (and thus prevented thickened cream from collecting under the lid or elsewhere and so escaping further agitation). With this device samples as small as 200 ml. could be churned satisfactorily. If the cream had been suitably cooled and had a fat content of 35–40%, the churning was usually complete in less than 1 min.

A detailed description of the apparatus in a size suitable for 500 ml. quantities of cream is as follows:

The cream is churned in a 1200 ml. stainless steel beaker (4 in. diameter, $6\frac{1}{2}$ in. high) by means of a beater directly attached to a $\frac{1}{4}$ h.p. electric motor (1425 r.p.m.). The motor (4) (see Fig. 1) is mounted, with its shaft vertical, on a wooden stand (1). The beaker (9) rests on a movable shelf (3) which may be slipped out to allow the beaker to be lowered clear of the agitator. The beaker is centred by means of a V-block (2) against which it is held by a spring-tensioned strap (8).

A lid (6) provides a loose bearing for the beater shaft and prevents escape of cream from the beaker. It is made from stainless steel sheet assembled by soldering, and is fitted with a soft rubber gasket (10) $\frac{1}{4}$ in. thick. The lid is secured by a cross-bar (11) pressed down by two wing nuts on $\frac{1}{4}$ in. threaded rods attached to the shelf (3).

The beater (7) is fabricated from stainless steel rod joined with 'Easyflo'. The central shaft is of $\frac{5}{16}$ in. diameter and the remainder $\frac{1}{8}$ in. The dimensions of the beater should be such that the clearances between it and the sides, top and bottom of the beaker do not exceed $\frac{1}{8}$ in.

The beater is connected to the motor by coupling (5) bored to accept the motor shaft ($\frac{1}{2}$ in.) and the beater shaft ($\frac{5}{16}$ in.), and is secured by two set-screws.

Photographs of the apparatus showing the individual parts and assembled as in use are given in Pl. 1 (*a*) and (*b*) respectively.

Where some of the samples to be churned are too small for the 500 ml. apparatus, a second beater and lid to fit a smaller beaker may be provided. These parts may be used

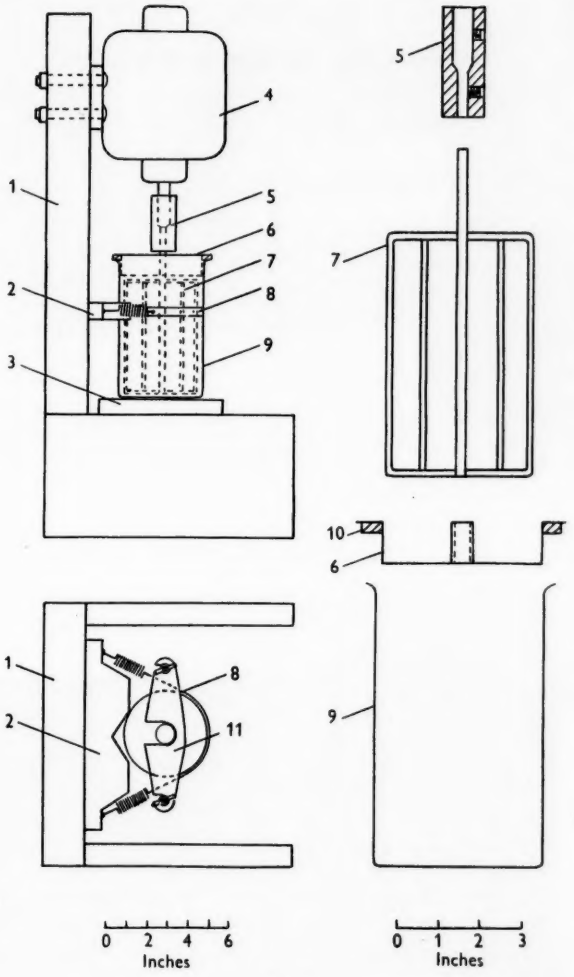



Fig. 1

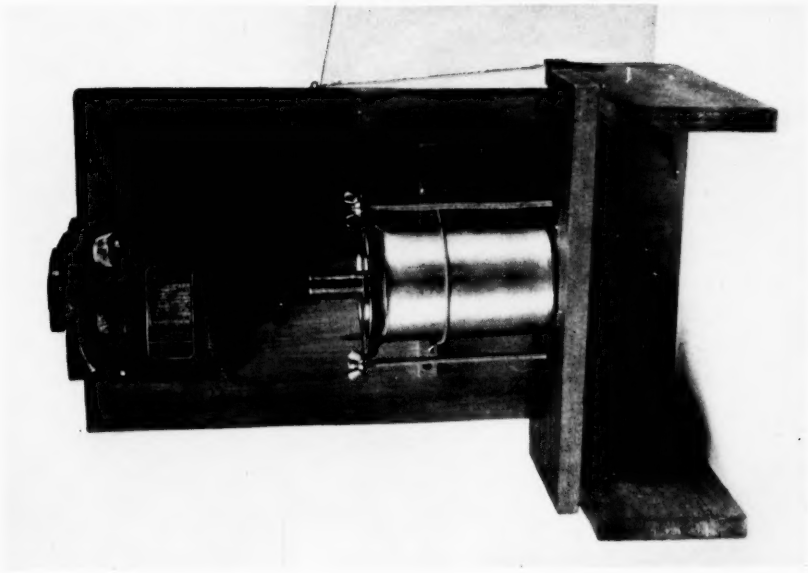
interchangeably with the larger ones if some provision is made for packing out or adjusting the V-block (2).

For preparation of butterfat, the cream is churned to large granules which are washed in the beaker. The butter is worked with pats to a sufficient extent to remove excess water before it is melted down. With this apparatus one operator can churn and work nine or ten samples per hour.

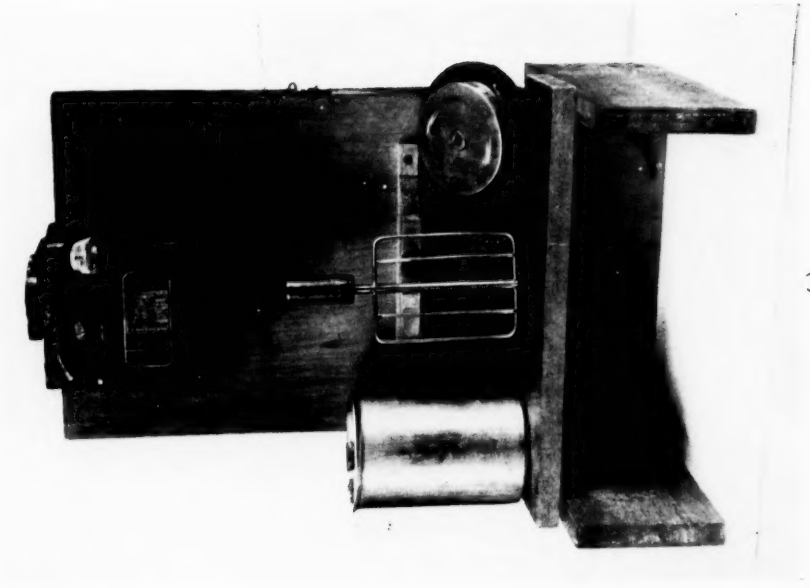
SUMMARY

Cream samples (200–500 ml.) are churned in about 1 min. in a covered beaker by an agitator directly coupled to a $\frac{1}{4}$ h.p. electric motor. The beater sweeps the whole of the enclosed space so as to prevent thickened cream from collecting in places where it can escape agitation.

(*MS. received for publication* 28 *July* 1953)



(b)



(a)

637.222.5:612.392.01

# 541. A NOTE ON THE VITAMIN D CONTENT OF INDIAN BUTTERS

By K. M. HENRY and S. K. KON

*National Institute for Research in Dairying, University of Reading*

Dikshit & Ranganathan (1) have reported very high values for the vitamin D content of south Indian butters and ghees, 6–18 i.u. vitamin D/g. fat in measurements on the non-saponifiable residue of the fat and 10–32 i.u./g. fat in measurements on the fat itself. The authors point out that these values are very much higher than any so far reported (2), the highest value for butter purchased in England being 4 i.u./g. fat (3); they attribute these high values to the strength of the sun to which cows and fodder are exposed in southern India.

Not only are these values very high but, in two samples where measurements were done on both the fat and its non-saponifiable residue, the discrepancy (4) between the two values was very much greater than we would expect, as experiments in this laboratory have shown that the discrepancy decreases as the potency of the sample increases (5,6,7,8).

As we have been interested for a considerable number of years in factors affecting the vitamin D content of butter we, like the Indian workers (1), were surprised at the exceptionally high potencies reported by them. Through the courtesy of Dr K. C. Sen, Director of Dairy Research, Indian Dairy Research Institute, Bangalore, we obtained two samples of butterfat from the milk of the herd of that Institute and one from the Agricultural Dairy Farm, Coimbatore, i.e. the farm from which the very potent samples (1) were obtained. In addition Dr Sen, in consultation with Dr V. N. Patwardhan of the Nutrition Research Laboratory, Indian Medical Research Council, Coonoor, kindly arranged that the Coimbatore and second Bangalore samples should be tested also at Coonoor. It was hoped to publish the results from both laboratories together. Owing to technical difficulties, however, no satisfactory results were obtained at Coonoor and work on other samples is in progress there. In the meantime it was agreed that our findings should be published separately.

## EXPERIMENTAL

### *Butters*

Butters were churned from milk collected in June 1951 (Bangalore, sample 1) and in April 1952 (Bangalore, sample 2; Coimbatore). The rendered fat was sent to this country by air and stored at 0° C. until used in the tests.

### *Experimental technique*

The butters were assayed by the prophylactic method used in this laboratory (4,8,9). The fats were given at two and the international standard of vitamin D at three or four levels. Groups of twelve rats were used, all comparisons being made between litter-mates. The results were calculated according to the method of the British Standards Institution (10) modified for litter-mate comparisons.

## RESULTS

The results are given in Table 1. They show that the vitamin D potency of the samples tested was very much lower than the values previously reported for Indian butters (1). In fact, the values are well within the range of values for English summer butters previously tested at this Institute (8, 9, 11, 12). It should be remembered that the tests were done on

Table 1. *Vitamin D content of Indian butterfat*

| Sample | Date collected | Potency (i.u./g. fat) | True fiducial limits at $P = 0{\cdot}95$ (i.u./g. fat) |
|---|---|---|---|
| Bangalore 1 | June 1951 | 0·56 | 0·43–0·72 |
| Bangalore 2 | April 1952 | 0·41 | 0·32–0·55 |
| Coimbatore | April 1952 | 0·29 | 0·25–0·33 |

the fats themselves, so that the true vitamin D potency of the butters would be even lower (4). With a further sample of Bangalore butter, Patwardhan (13) has found a value of 1·2 i.u. vitamin D/g. fat, a value which, though higher, is of the same order as those now obtained and considerably lower than values previously reported from India (1). Further work is in progress in India (13).

## SUMMARY

1. The vitamin D potency of three samples of south Indian butter has been determined in prophylactic tests with rats.

2. Two samples of butter from Bangalore contained 0·56 and 0·41 i.u. vitamin D/g. fat, a sample from Coimbatore contained 0·29 i.u./g. fat.

## REFERENCES

(1) Dikshit, P. K. & Ranganathan, S. (1950). *Indian J. med. Res.* **38**, 37.
(2) Fixsen, M. A. B. & Roscoe, M. H. (1937–38). *Nutr. Abstr. Rev.* **7**, 823.
(3) Coward, K. H. & Morgan, B. G. E. (1935). *Brit. med. J.* ii, 1041.
(4) Booth, R. G., Henry, K. M. & Kon, S. K. (1942). *Biochem. J.* **36**, 445.
(5) Kon, S. K. & Booth, R. G. (1933). *Biochem. J.* **27**, 1302.
(6) Kon, S. K. & Booth, R. G. (1934). *Biochem. J.* **28**, 111.
(7) Kon, S. K. & Booth, R. G. (1934). *Biochem. J.* **28**, 121.
(8) Henry, K. M. & Kon, S. K. (1942). *Biochem. J.* **36**, 456.
(9) Kon, S. K. & Henry, K. M. (1935). *Biochem. J.* **29**, 2051.
(10) British Standards Institution (1940). Specification No. 911.
(11) Kon, S. K. & Henry, K. M. (1936). *Biochem. J.* **30**, 776.
(12) Campion, J. E., Henry, K. M., Kon, S. K. & Mackintosh, J. (1937). *Biochem. J.* **31**, 81.
(13) Patwardhan, V. N. (1953). Private communication.

(*MS. received for publication* 23 *July* 1953)

637.333.3:576.8:637.361.3

# 542. THE SURFACE MICROFLORA OF STILTON CHEESE—NORMAL AND SLIP-COAT

By MARJORIE BRINDLEY

*School of Agriculture, University of Nottingham*

## INTRODUCTION

Coat formation in Stilton cheese is started 5–10 days after manufacture by scraping the surface to fill in crevices and to smooth the coat. Normally the cheese, when ready for scraping, is firm at the surface, greasy to the touch and smells like ripe pears. In some dairies, however, an abnormal condition known as 'slip-coat' may be apparent at the scraping stage. The cheese is not firm to the exterior, but has a layer of softened material $\frac{1}{2}$–$\frac{3}{4}$ in. deep resembling a partially drained cream cheese. The texture is flaky, whereas in the scrapings from the surface of a normal cheese the texture is granular. Patches of grey, or, less commonly, yellow or brown discoloration, may be formed at the surface of slip-coat cheese.

Little information is now available on the factors involved in normal coat formation in Stilton cheese, but it may be that the formation of a normal coat is due mainly to the loss of moisture from the surface of the cheese and to the growth of micro-organisms, which may vary from cheese to cheese or from dairy to dairy. The coats may vary in colour from drab to a light golden brown or a bright orange brown. The surface may be dry and wrinkled, slightly moist and wrinkled, or smooth and wet. The thickness of the coats may also vary. The growth of micro-organisms will be influenced, either directly or indirectly, by the conditions at the surface of the cheese. The factors likely to affect the flora include the pH, the moisture content and the concentration of sodium chloride. The extent of protein breakdown and the mineral content of the surface layer may also have an effect. The growth of some micro-organisms will cause changes in some of these factors and thus influence the growth of other micro-organisms.

## LITERATURE

Percival & Mason (1) found that *Oospora lactis* was abundant on the coat of the cheese for the first 15 or 20 days when the surface was moist. Other moulds occasionally found on cheese of all ages were *Mucor mucedo*, *Aspergillus glaucus* and *Cladosporium herbarum*. A species of *Torula* was also regularly found at the surface and within the cheese. Hiscox, Harrison & Wolfe (2) found that the internal flora of the cheese in its early stages consisted almost entirely of streptococci, but after 7 days rod forms appeared and increased in numbers throughout the ripening period. They observed also that variations occurred in the flora of individual cheese, even where the same bulk of milk was used for manufacture. Davis (3), working with Cheddar cheese, showed that the zone (about $\frac{1}{4}$ in. wide) at the outside of the cheese and around cracks was in a more highly oxidized condition than the interior, and that many fault-producing micro-organisms, such as *Proteus*, chromogenic cocci and moulds, could grow in this zone. Davis's findings indicate that the surface of Stilton cheese provides more favourable conditions for the growth of micro-organisms than the interior, at least in the early stages of coat formation when the surface is moist.

Many workers have studied the flora of the coat of the surface-ripened cheese, particularly Limburger. Although Stilton and Limburger cheese are very different, certain conditions of manufacture are similar, and in the early stages the two varieties have some characteristics in common, such as pH, a similar basic medium and moisture content. Laxa (4), studying the surface flora of Harrach and Knoppist cheese, found *Oospora*, yeasts and lactic acid bacteria. Wolff (5) isolated nine different pigment-forming bacteria from surface smears of Tilsiter, Romadour and Woringer cheese. One of these organisms (no. IX), *Bacterium linens*, was studied further (6). Later descriptions of *Bact. linens* by Steinfatt (7) suggest that it is a member of the genus *Corynebacterium*. Kelly (8), examining Limburger cheese by means of contact slides, found that a definite sequence of micro-organisms occurred. Cocci were replaced by yeasts, which after 6 or 7 days were succeeded by short slender rods resembling *Bact. linens*. *Oospora* was found only occasionally, but when it was present in large numbers the cheese developed a wrinkled surface. Kelly & Marquardt (9) showed that the pH increased more rapidly at the surface than in the interior of Limburger cheese. They believed that yeasts, using protein and lactic acid, increased the pH and encouraged the growth of *Bact. linens*.

Macy & Erekson (10), examining the surface slime of Roquefort, Port de Salut, Tilsit and Limburger cheese, found that changes in pH followed the sequence of micro-organisms. In later work they (11) found that large numbers of *Streptococcus lactis* were present on the surface of Roquefort cheese before salting. After salting, the streptococci were replaced by rod forms and yeasts. At 3 weeks old, when the pH, following the growth of yeasts, had risen to 5·9, rod forms began to appear and quickly outgrew the yeasts. After 6 weeks the pH had reached 7·3, and *Torula*, rods, cocci and *Penicillium roqueforti* were present. With Limburger cheese Yale (12) found that bacteria succeeded yeasts after about 4 days, and reached their maximum numbers at 14 days. The predominating organisms on the cheese surface at 2–28 days old were Gram-positive, aerobic non-sporing rods. *Bact. linens* was also present in large numbers.

'Slip-coat' is said to be the result of moisture collecting at the surface, or under the rind, of the cheese (13, 14) because of faulty manufacture and storage. The factors believed to be involved are inadequate control of ventilation during the drainage and coating periods, insufficient turning of the cheese, and infrequent changing of the bandages (13, 14, 15). Bryant & Hammer (16), examining Roquefort cheese, described a soft-edge defect which resembled slip-coat in Stiltons. They attribute the soft edge mainly to the accumulation of moisture at the edges of the cheese.

The investigation described in this paper was designed to provide information on some of the conditions found at the surface of both normal and slip-coat Stilton cheese at the scraping stage. Information on the following points was required for both types of cheese: the numbers and types of micro-organisms present, the pH values and the moisture contents.

## MATERIALS AND METHODS

### *The sample*

The samples of both normal and slip-coat cheese consisted of the surface layer of the cheese removed with a sterile knife. The samples were obtained immediately before the manufacturer carried out the normal process of scraping. For a complete examination

of normal cheese, scrapings from a number of cheese were aggregated to form each sample. Where a complete examination of the flora was being carried out, the sample, ½–1 lb., consisted of the scrapings from twenty to thirty cheese. Six composite samples of normal cheese were obtained in this way. Twelve samples were also taken from eight individual cheese for a comparison of the types of organisms present. A qualitative but not quantitative examination of the flora of these individual samples was carried out.

The slip-coat cheese were sampled individually; the weight of each sample was approximately equal to that of the composite samples taken from the normal cheese.

The samples were examined by several methods; microscopic examination of direct smears from the sample, plate counts of suspensions, microscopic smears of the first suspension of the sample, and determinations of the pH and of the moisture contents.

To compare the pH's and the counts of micro-organisms in the interior of the cheese with those at the surface, a number of samples, each consisting of two cores from a cheese, were also taken. With the normal cheese, three samples were examined for total counts and eight samples for pH. The pH of the interior of one slip-coat sample was determined.

### *Preparation of the cheese suspension*

Cheese suspensions were made by a modification of Harrison's method (17). The cheese samples were mixed by grinding in a sterile mortar. From each ground sample 10 g. was weighed in a sterile weighing bottle, and transferred to a large wide-necked bottle containing 90 ml. of sterile 2% sodium citrate solution at 50° C. Glass beads were added to the sodium citrate solution to assist in breaking up the cheese. After shaking 50 times the sample was placed in a 50° C. water-bath for 15 min. The suspension was then inverted several times and further dilutions up to $10^{-8}$ made in a 2% sodium citrate solution.

### *Plating*

Plate counts were made on (*a*) yeast-glucose agar (pH 6·8), for lactic acid bacteria and the approximate total count; (*b*) nutrient agar (pH 7·0), for miscellaneous organisms; and (*c*) beerwort agar (pH 5·0), for yeasts and moulds. All media except the beerwort agar, which was incubated at 22° C. for 7 days, were incubated at 30° C. After counting, colonies were picked from each plate by Harrison's method (17) and transferred to dextrose Lemco broth; after incubation the cultures were streaked on agar slopes and then purified by repeated restreaking on yeast-glucose agar.

### *Microscopic examination*

Direct smears were made from the cheese samples by spreading a loopful of cheese over the surface of a slide and staining by Newman's method.

### *Determination of* pH *values*

One gram of cheese was ground with 10 ml. of cooled, boiled, glass-distilled water and pH's determined with a glass electrode.

### *Estimation of moisture content*

Moisture was determined by the British Standards Institution method (18).

### *The surface flora of normal cheese*

In Table 1 the results of plate counts of the surface scrapings of a series of samples are summarized.

Table 1. *Plate counts of surface scrapings (millions per gram)*

| Sample no. | Age of cheese in days | Yeast-glucose agar | | Nutrient agar | | Beerwort agar | |
|---|---|---|---|---|---|---|---|
| | | Bacteria and yeasts | Moulds | Bacteria and yeasts | Moulds | Bacteria and yeasts | Moulds |
| V7 | 8 | 5,960 | <1 | 5,490 | <1 | 4,220 | <1 |
| Y8 | 7 | 3,460 | 1 | 3,230 | 10 | 1,790 | 10 |
| L1 | 7 | 8,000 | 30 | 3,200 | 20 | * | * |
| L2 | 8 | 3,190 | 10 | 1,390 | <10 | 165 | 10 |
| L3 | 10 | 8,600 | 30 | 1,890 | 10 | 1,100 | * |
| X1 | 5 | 17,100 | 400 | 15,100 | 300 | * | * |

* Plates overgrown with moulds.

The surface layer of Stilton cheese at the time of scraping clearly has a very high count. The counts of bacteria and yeasts on yeast-glucose agar, however, are very variable, ranging from 3190 millions per gram in sample L2 to 17,100 millions per gram in sample X1. In general, the counts on yeast-glucose agar are only slightly higher than those on nutrient agar, although samples L1, L2 and L3 show considerably higher counts on yeast-glucose agar.

The high count in sample X1 may be due to the fact that the cheese were only 5 days old when scraped. Hiscox *et al.* (2) found that at 3 or 4 days the bacterial count of the interior of a Stilton cheese is at its maximum and at 5 days the numbers of bacteria have declined only slightly. Cultural examinations of the types of bacteria in the surface scrapings of X1 confirmed the view that the surface and interior floras are similar (Table 4). Owing to the vigorous mould growth on beerwort agar, with samples L1 and X1, the counts of only four samples (V7, Y8, L2 and L3) are available, and in three instances the counts of bacteria and yeasts on beerwort agar were considerably lower than those on yeast-glucose agar. In two instances the results were also considerably lower than those on nutrient agar. Comparison of the counts on beerwort agar with the pH's of the samples given in Table 3 shows that the majority of micro-organisms in sample V7, the pH of which was 5·5, could grow on beerwort agar at pH 5·0.

Although the results of the mould counts on beerwort agar are incomplete, those on yeast-glucose agar show the extent of mould of the surface layer of the cheese. Moulds were present in very much smaller numbers than bacteria or yeasts, but these counts also varied from sample to sample.

As a basis for comparison of the internal counts with those at the surface of the cheese, two cores were taken from each of three cheese selected at random from the group of cheese from which sample L2 was taken. Each sample, L2(1), L2(2) and L2(3), was examined by the same technique as the surface scrapings. The results of the plate counts are given in Table 2.

These results show that, at the time of scraping, the counts of micro-organisms at the surface were higher than those inside the cheese. Allowing for variations in individual cheese, and for the differences in the technique and media used, these findings are of the same order as those of Hiscox *et al.* (2).

Table 2. *Total counts of micro-organisms in interior of cheese (millions per gram)*

| Sample no. | Yeast-glucose agar | Nutrient agar | Beerwort agar |
|---|---|---|---|
| L2(1) | 245 | 214 | 79 |
| L2(2) | 640 | 321 | No result |
| L2(3) | 177 | 123 | 36 |

The counts of the surface scrapings of the cheese appear to bear little relation to the pH's (Table 3). It might be expected that a lower pH would restrict the growth of micro-organisms and result in a lower count. The results show, however, that a low pH, such as V7 and X1, does not reduce the count. Results given later suggest that the pH at the surface influences the types, rather than the numbers, of organisms in the scrapings.

Table 3. pH *Values of Stilton cheese*

| Surface scrapings | | Interior | |
|---|---|---|---|
| Sample no. | pH | Sample no. | pH |
| V7 | 5·5 | I1 | 4·9 |
| Y8 | 6·2 | I2 | 5·0 |
| L1 | 6·5 | I3 | 4·8 |
| L2 | 6·7 | I4 | 5·2 |
| L3 | 6·8 | I5 | 4·7 |
| X1 | 5·9 | I6 | 4·9 |
| | | I7 | 4·9 |
| | | I8 | 5·3 |

The pH of the surface at scraping does not appear to be related to the development of acidity in the curd at the time of moulding. At dairies V and L, the titratable acidities in the curd at moulding were similar, but the pH values at the surface of 8-day-old cheese varied considerably. Another interesting feature of the results is that all the pH values are higher than those found in the interior of a normal Stilton cheese at the same age. Mattick (19), examining experimental Stilton cheese, found that the pH values for the interior of the cheese were 4·59 at 3 days and 4·78 at 7 days. It appears, therefore, that the increase in pH is more rapid at the surface than in the interior of the cheese. Similar differences in pH values of the surface smear and the interior of the cheese have been found in Limburger and other ripened soft cheese. This has been attributed to the growth of yeasts which increase the pH, and allow the alkali-producing bacteria to grow.

There are two possible explanations of the present results. First, that there are differences in chemical composition between the surface layers and the interior of the cheese and, secondly, that the larger numbers and different types of micro-organisms at the surface layer of the cheese increase the pH more rapidly than inside the cheese.

### *Microscopic appearance of cheese samples*

Yeasts, usually of the oval-celled budding type, were common to all the samples examined. The other types of micro-organisms present varied from sample to sample. At the lower pH values the flora consisted mainly of oval yeasts, diplococci, short chains of cocci, and short or long rods growing in clusters or in V formations. As pH values increased, rods of various types occurred in increasing numbers; yeasts, diplococci, short chains and groups of cocci were also found.

*Cultural examination of micro-organisms*

The predominant organisms isolated from the various media are given in Table 4. Moulds have been omitted from the table, but the main types found are mentioned later.

Table 4. *Types of bacteria in surface scrapings*

| Sample no. | Medium | Total no. of colonies examined | Yeasts | Lacto-bacilli | Strepto-cocci | Pig-mented cocci | Aerobic spore-formers | Diph-theroid rods | Coli-forms |
|---|---|---|---|---|---|---|---|---|---|
| Y7 | Nutrient agar | 10 | 4 | 4 | 0 | 0 | 0 | 2 | 0 |
| | Yeast-glucose agar | 17 | 6 | 11 | 0 | 0 | 0 | 0 | 0 |
| | Beerwort agar | 12 | 3 | 9 | 0 | 0 | 0 | 0 | 0 |
| Y8 | Nutrient agar | 17 | 3 | 0 | 8 | 0 | 0 | 5 | 1 |
| | Yeast-glucose agar | 12 | 4 | 5 | 1 | 2 | 0 | 0 | 0 |
| | Beerwort agar | 14 | 7 | 5 | 1 | 1 | 0 | 0 | 0 |
| L1 | Nutrient agar | 10 | 0 | 0 | 0 | 0 | 8 | 2 | 0 |
| | Yeast-glucose agar | 11 | 0 | 0 | 0 | 2 | 7 | 2 | 0 |
| | Beerwort agar | 10 | 4 | 6 | 0 | 0 | 0 | 0 | 0 |
| L2 | Nutrient agar | 13 | 0 | 2 | 0 | 3 | 5 | 3 | 0 |
| | Yeast-glucose agar | 13 | 0 | 2 | 0 | 1 | 8 | 2 | 0 |
| | Beerwort agar | 13 | 3 | 4 | 1 | 3 | 2 | 0 | 0 |
| L3 | Nutrient agar | 9 | 0 | 0 | 0 | 1 | 6 | 2 | 0 |
| | Yeast-glucose agar | 10 | 1 | 2 | 0 | 4 | 3 | 0 | 0 |
| | Beerwort agar | 11 | 5 | 5 | 0 | 0 | 1 | 0 | 0 |
| X1 | Nutrient agar | 12 | 6 | 0 | 4 | 0 | 0 | 0 | 2 |
| | Yeast-glucose agar | 10 | 2 | 0 | 7 | 1 | 0 | 0 | 0 |

These results show that yeasts form an important part of the surface flora of Stilton cheese during the early stages of coat development. They are the only type of organism appearing in each of the six samples examined. Their presence confirms the microscopic findings in the cheese and cheese suspensions.

Lactobacilli predominated in all the samples except no. X1, which, however, was only 5 days old when examined. At this stage the numbers of lactobacilli at the surface of the cheese would probably be too low to be detected by the methods used. The results suggest that lactobacilli replace streptococci more quickly at the surface than in the interior of the cheese. During the drainage period, as whey leaves the cheese, the soluble mineral salts move to the outside of the cheese where they are concentrated. Hunter (20) has shown that the growth of lactobacilli is encouraged by the addition of minerals to a basic cheese medium. The growth of lactobacilli at the surface of Stilton cheese during the drainage period may, therefore, be stimulated by the mineral content of the surface layers.

Samples L1, L2 and L3 were made from milk heated to 162° F. for 15 sec. Slatter & Halvorsen (21) examined the heat resistance of lactobacilli isolated from Cheddar cheese, and found that approximately 48% of those found in raw-milk cheese survived heating to 160° F. for 15 sec. Lactobacilli have been isolated from the interior, as well as the surface of Stilton cheese made from pasteurized milk (22). The lactobacilli found in Stilton cheese made from pasteurized milk may be strains which have survived the heat treatment of the milk, but it is probable that some of them were contaminants which gained access to the milk and curd during manufacture. The pH of 5·5 of sample V7 is suitable for the growth of lactobacilli. The remaining samples, however, had higher pH's (Table 3), which may account for the wider variety of bacteria present. The majority of the lactobacilli grew well in litmus milk, producing an acid clot usually without reduction.

Davis(23) also found that lactobacilli isolated from immature Cheddar cheese did not reduce litmus.

An interesting feature of samples L1, L2 and L3 is the presence, in large numbers, of aerobic spore-forming rods of the *Bacillus cereus-megatherium* group(24), which were not found in any other sample. The pH's of the samples, ranging from 6·5 to 6·8, would permit rapid development of the spore-formers. These bacteria probably originated from the raw milk or from plant, small numbers in the sporing state resisting the heat treatment of the milk.

The comparatively large numbers of yellow- and orange-pigmented bacteria in samples L1, L2 and L3 were of interest, as many of the cheese at dairy L developed the wet orange type of coat previously described. The pigmented cultures were either cocci, of the genera *Micrococcus* and *Sarcina*, or diphtheroid rods. Diphtheroid rods, forming cream-coloured colonies, were found in samples V7 and Y8, but none was detected in sample X1. It appears from the results obtained that diphtheroid rods, pigmented or non-pigmented, are common members of the surface flora of normal Stilton cheese. The eighteen cultures isolated showed distinct diphtheroid morphology and, although variations occurred in their characteristics, they are regarded tentatively as members of the genus *Corynebacterium*.

Eighteen cultures of Gram-positive, catalase-positive, pigmented cocci were isolated from the samples. The majority (thirteen cultures) were species of *Sarcina*, either *S. citrea* or *S. aurantiaca*, while the remaining cultures were micrococci, either *Micrococcus aurantiacus* or *M. freudenreichii*.

Samples Y8 and X1, obtained from raw-milk cheese, contained streptococci. A small amount of starter was used in the manufacture of the cheese from which sample X1 was obtained, and the streptococci, which belonged to the *lactis-cremoris* group, probably originated mainly from that source. Their appearance in sample X1 may be due to the fact that the cheese were only 5 days old when scraped.

Sample Y8 is different, however, in that no starter was used in its manufacture, and the streptococci presumably originated from the raw-milk supply or from the cheese-making equipment. As many of the cheese at the dairy from which the sample was obtained showed signs of weakness and a lack of acidity, it seems likely that acid production in the cheese was slow in the early stages of manufacture and storage. Further examination of the cultures showed that some of them were enterococci, of the *Streptococcus faecalis* type, which are known to survive for long periods in blue-veined and other varieties of cheese. It was also found that if the early stages of acid production are slow, the scraping stage is late in developing. Although the cheese from which sample Y8 was taken were 7 days old when scraped, they showed little greasiness at the surface.

Coliform organisms in small numbers were present in the raw-milk cheese samples (Y8 and X1), but were not present in significant numbers in any of the scrapings from cheese made from pasteurized milk.

The results show that yeasts and lactobacilli, with smaller numbers of diphtheroid rods, are found regularly at the surface of Stilton cheese. Where, however, the cheese are scraped at an early age, or are late in maturing, streptococci may take the place of lactobacilli. Aerobic spore-formers, pigmented cocci and coliform organisms occur irregularly and in variable numbers. The results also show that marked differences may exist in the surface flora of Stilton cheese made from pasteurized milk at different dairies.

### *Minority flora of scrapings*

The organisms appearing in Table 4 represent the majority flora of the cheese surface. Visual examination of the lower dilutions in a series of plates indicated, however, that other colony types were present in small numbers in the samples. These colonies were examined microscopically and were found to be oval yeasts showing mycelial threads, pink oval yeasts, pigmented cocci and Gram-negative rods.

The moulds occurring on the plates in variable numbers (see Table 1) were examined macroscopically, under a low-power objective (Table 5).

Table 5. *Moulds in surface scrapings*

| Sample no. | Moulds present |
|---|---|
| V7 | *Oospora* in small numbers |
| Y8 | *Oospora* in small numbers |
| L1 | *Mucor* and *Penicillium* sp. |
| L2 | *Mucor* |
| L3 | *Penicillium*, *Mucor* and *Oospora* in small numbers |
| X1 | *Penicillium* sp. |

Owing to the nature of their colonies the moulds were sometimes picked from the plates together with the bacteria and yeasts, but Table 1 shows that they were not present in any sample in numbers sufficiently large to be recorded in the statistical pick results. *Oospora*, *Mucor* and *Penicillium*, however, each occurred in three samples, although the numbers of *Oospora* were always low. These results differ from those of Percival & Mason (1), who found that *O. lactis* was abundant on the surface of Stilton Cheese for the first 15–20 days. Other moulds isolated by Percival & Mason were *Mucor mucedo*, *Aspergillus glaucus* and *Cladosporium herbarum*; species of the last two genera, however, were not isolated in this work.

### *Litmus-milk reactions*

The cultures isolated from the cheese, and recorded in Table 4, were inoculated into litmus milk to obtain an indication of the changes which may be produced at the surface of the cheese. The cultures were incubated at 30° C. for 28 days, examined daily for 7 days, and then at weekly intervals. As the surface scrapings were taken from cheese 5–10 days old, the changes occurring in litmus milk after 14 days' incubation are given in Table 6, since the changes which take place during this time are most likely to be significant.

Table 6. *Reactions in litmus milk by cultures isolated from surface scrapings*

| Litmus milk reactions 14 days at 30° C. | No. of cultures | | | | | |
|---|---|---|---|---|---|---|
| | V7 | Y8 | L1 | L2 | L3 | X1 |
| Total cultures examined ... | 39 | 42 | 31 | 39 | 30 | 22 |
| Acid and coagulation | 23 | 19 | 7 | 10 | 7 | 14 |
| Acid, no coagulation | 6 | 11 | 5 | 5 | 8 | 7 |
| No visible change | 1 | 1 | 2 | 2 | 2 | 1 |
| Sweet clot, little or no change | 4 | 3 | 0 | 0 | 0 | 0 |
| Alkalinity | 2 | 6 | 1 | 4 | 0 | 0 |
| Marked digestion | 0 | 2 | 16 | 18 | 13 | 0 |
| Slight digestion | 3 | 0 | 0 | 0 | 0 | 0 |

The table indicates the different reactions which may be produced in litmus milk by the growth of bacteria and yeasts found at the surface of Stilton cheese. It is interesting that

in samples V7 and X1 the predominant change was acid production, whereas in samples L1 and L2 there was a slightly greater number of alkali and digesting organisms than acid producers. In sample L3 also, the numbers of digesting bacteria were high. Sample Y8 contained predominantly acid producers, but also a considerable number of alkali and sweet curdling organisms. Comparison of these results with the pH's of the cheese (Table 3) indicates that, in these samples, a higher pH at the surface of the cheese is associated with an increase in the number of organisms producing alkalinity. It may be that the growth of alkali-producing micro-organisms at the surface of Stilton cheese is one factor responsible for the more rapid increase in pH which occurs at the surface as compared with the interior.

*Qualitative analysis of scrapings from individual cheese*

A further twelve samples of scrapings from normal cheese at dairy D were examined for their microscopic appearance, pH's and moisture contents. Also the predominating micro-organisms were isolated from the cheese (without reference to their numbers) by inoculating a loopful of the sample into broth, from which streak plates were prepared and colonies isolated.

Individual cheese at a dairy may show variations in the type of coat formed, even though the methods of handling and storing the cheese are identical. The object of this examination, therefore, was to study the characteristics at the surface of individual Stilton cheese and to observe the type of coat which developed as the cheese matured. Information on the moisture content was also required.

All the samples were obtained from cheese made from pasteurized milk to which starter was added at the rate of 10 ml. to 12 gal. of milk. The samples had a granular texture and yeasty smell when examined. Although the samples were normal at the time of scraping,

Table 7. pH*'s and moisture contents of samples of surface scrapings*

| Sample no. | Titratable acidity at moulding curd | Age in days | Description of sample | pH | Moisture-content percentage |
|---|---|---|---|---|---|
| D12 | 0·94 | 10 | First scraping | 6·0 | 52·28 |
| D13 | 0·98 | 9 | First scraping | 5·7 | 55·50 |
| D14 | 0·90 | 8 | First scraping | 5·1 | 54·78 |
| D15 | 1·08 | 9 | First scraping | 5·3 | 58·40 |
| D16 | 0·97 | 8 | First scraping | 5·6 | 43·30 |
| D17 | 0·93 | 10 | First scraping | 5·5 | 42·80 |
| D18(1) | 0·90 | 9 | First scraping | 4·9 | 58·70 |
| D18(2) | | 10 | Second scraping of cheese 18 | 5·4 | 57·50 |
| D18(3) | | 11 | Third scraping of cheese 18 (hoop removed) | 5·7 | 55·20 |
| D19(1) | 0·90 | 9 | First scraping | 4·9 | 56·60 |
| D19(2) | | 10 | Second scraping of cheese 19 | 5·3 | 56·80 |
| D19(3) | | 11 | Third scraping of cheese 19 (hoop removed) | 5·8 | 56·35 |
| V7 | — | 8 | Composite | | 52·83 |
| Y8 | — | 7 | Composite | | 51·50 |

most of the cheese formed coats slowly and were moist at the surface after 3 weeks' storage. As there was no detectable sign of slip-coat at scraping, the results are recorded with those of the normal samples. Additional data concerning the samples, together with the results of pH and moisture-content determinations, are summarized in Table 7. The moisture contents of the composite samples of V7 and Y8 are also given.

*Microscopic appearance of samples*

The eight cheese examined had a similar surface microflora at the time of scraping. Cultural examination of the organisms isolated from the samples showed that the predominating bacteria were lactobacilli producing an acid clot in litmus milk. In the cheese the cultures produced mainly short, thick rod forms, while longer cells, frequently curved and twisted, and in characteristic formations were formed on yeast-glucose agar. Other organisms found were yeasts growing in clusters, surrounded by or interspersed with rods, and mould hyphae. The examination of samples D18 and D19 suggests that lactobacilli are present first, and that yeasts increase rapidly at the surface of Stilton cheese at about the time when scraping is carried out. The yeasts may then become predominant. The number of samples examined is too small, however, to warrant definite conclusions as to the exact course of development of micro-organisms at the surface of the cheese.

Observations made after the cheese had been scraped showed that, with one exception, coat formation was slow. Instead of drying out, some parts of the surface of the cheese were very moist, whereas others formed a heavy coat. This was not exclusively related to the moisture content at scraping. Sample D14, which had a comparatively high moisture content (54·78%), dried out and coated normally, whereas sample D17, with the lowest moisture content of the series (42·80%), remained wet and was slow in forming a coat. During the drainage period and until the cheese were 3 or 4 weeks old they were stored in a fairly humid atmosphere with poor ventilation. On removal to a dry atmosphere the moist surfaces disappeared and coat formation became normal. The coats formed on the cheese (samples D12–19) were all very similar, being greyish brown, dry and wrinkled. The flora of sample V7 was very similar to that of samples D12–19, and the coats formed on the cheese at dairy V were also of a similar type.

*Influence of flora on coat formation*

The limited results available suggest that the development of a dry, wrinkled, greyish brown coat on Stilton cheese is associated with a relatively low pH at the time of scraping, and with a flora consisting mainly of yeasts and lactobacilli. In comparison, the formation of a bright orange, moist coat appears to be associated with higher pH values and a wider variety of micro-organisms, which may include aerobic spore-formers, pigmented cocci and corynebacteria, in addition to lactobacilli and yeasts. The pigmented cocci and corynebacteria have been shown to produce an orange coloration on soft cheese, but the part played by the aerobic spore-formers in the formation of the coat has not been determined.

*The surface flora of slip-coat cheese*

Manufacturing data relating to the three slip-coat samples examined are given in Table 8.

The results of plate counts of the samples of slip-coat cheese are set out in Table 9. Each sample represents the scrapings from one cheese.

Comparison of these results with those of normal cheese (Table 1) shows that the counts of bacteria and yeasts in the slip-coat cheese samples are not higher and may be lower than those of the normal cheese. In two instances (DS1 and X2) the mould count is similar to that of the average normal cheese. Sample X2, however, has a considerably

Table 8. *Data relating to slip-coat samples*

| Sample no. | Milk | Month of manufacture | Starter | Acidity at moulding | Slip-coat development | Age of cheese in days | Other observations |
|---|---|---|---|---|---|---|---|
| DS1 | Raw | April | None | Not recorded | Pronounced | 7 | Three cheese were manufactured on the same day from different supplies of milk, but only one (DS1) developed slip-coat, although all were stored under the same conditions. After scraping the slip-coat cheese was removed to a cool dry cellar. A thickened coat developed with no further evidence of slip-coat. Internal discoloration of the cheese occurred during ripening |
| D11 | Bulked pasteurized | September | 10 ml. to 12 gal. milk | 0·98% | Pronounced | 8 | One of two cheese made from the same bulk of milk. A total of twelve cheese were draining at the time, but only one developed slip-coat |
| X2 | Bulked raw milk 300 gal. | July | Very small quantities | Not recorded | Fairly pronounced | 6 | Bulked raw milk was manufactured in two vats. The cheese from one vat were normal whereas those from the other showed slip-coat. After thorough scraping, coat formation and ripening proceeded normally |

Table 9. *Plate counts of surface scrapings (millions per gram)*

| Sample no. | Age of cheese in days | Yeast-glucose agar | | Nutrient agar | | Beerwort agar | |
|---|---|---|---|---|---|---|---|
| | | Bacteria and yeasts | Moulds | Bacteria and yeasts | Moulds | Bacteria and yeasts | Moulds |
| DS1 | 7 | 610 | 20 | 494 | 15 | 540 | 20 |
| X2 | 6 | 11,000 | 10 | 8,520 | 10 | — | — |
| D11 | 8 | 13,960 | 960 | 9,270 | 890 | 11,080 | 1,240 |

lower count of moulds than the normal sample X1, although both samples were obtained from the same dairy. The mould count of sample D11 is greater than that of any sample of the normal cheese scrapings, and is significantly higher than five out of the six samples.

The pH's of the three samples of slip-coat scrapings and of the two cores from sample DS1 are given in Table 10.

The pH's of samples DS1 and X2 are similar to the normal samples V7 and X1, and are lower than those of samples Y8, L1, L2 and L3. Before any pH values for the scrapings of normal cheese had been obtained it was thought that the proteolytic action of the bacteria found in the samples of slip-coat cheese might lead to an increase in the pH at the surface. These results suggest, however, that at least at the time of scraping, the presence of proteolytic bacteria does not effect an abnormal increase in pH at the surface of slip-coat cheese. The remaining sample D11 has a pH of 7·3 which is higher than that of any sample of normal cheese, and can be partly attributed to the growth of large numbers of moulds at the surface.

### *Microscopic appearance of cheese samples*

The three samples contained oval-celled budding yeasts, but the remaining flora varied in each sample. The only outstanding differences from the normal samples were that the rods were isolated and were not closely associated with the yeasts, and the large numbers of mould hyphae in one sample (D11).

### *Types of micro-organisms*

The outstanding feature of the results given in Table 11 is the presence of *Proteus vulgaris* in two of the slip-coat samples (DS1 and X2). These organisms were not found in any of the samples of normal cheese scrapings (Table 4).

The streptococci in sample X2, with two exceptions, were of a different type from those isolated from the slip-coat samples DS1 and D11, and from the normal cheese samples. The streptococci from X2 gave an acid clot, quickly followed by partial digestion, in litmus milk and were identified as *Streptococcus liquefaciens*.

The other types of bacteria present, namely, the lactobacilli, micrococci, sarcinae, corynebacteria and coliforms, were all found in one or more of the samples of normal cheese. Yeasts of similar types were common to both the slip-coat and normal samples.

The moulds occurring on the plates of sample DS1 were species of *Mucor* and *Oospora*, while those in X2 were *Penicillium* sp. Similar types of moulds in similar numbers were found in the normal cheese scrapings. In considering the results of sample D11, however, it must be borne in mind that there was a mould count of 1240 millions per gram on beerwort agar. The moulds isolated were all of the *Oospora lactis* type. Such a high count of *O. lactis* was not found in any of the samples of normal cheese examined, and it must, therefore, be regarded as abnormal. Percival & Mason (1913) found, however, that *O. lactis* was very common on the surface of Stilton cheese in the early stages after manufacture.

Attention may be drawn, therefore, to three types of organisms found in the surface flora of the slip-coat samples but not demonstrated in the samples of normal cheese examined. They are *Proteus vulgaris*, common to samples DS1 and X2, *Streptococcus liquefaciens* in sample X2, and *Oospora lactis* in sample D11.

Table 10. pH's *of surface scrapings*

| Sample no. | pH | |
|---|---|---|
| | Scrapings | Interior |
| DS1 | 5·8 | 4·9 |
| X2 | 5·8 | — |
| D11 | 7·3 | — |

Table 11. *Predominating micro-organisms in slip-coat samples*

| Sample no. | Medium | Total no. of colonies examined | Yeasts | Lacto-bacilli | Diphtheroid rods | Pigmented cocci | Coli-forms | Strepto-cocci | *Proteus vulgaris** | Gram-negative alkali-producing rods | *Oospora*† |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DS1 | Nutrient agar | 11 | 0 | 2 | 0 | 0 | 5 | 0 | 4 | 0 | 0 |
| | Yeast-glucose agar | 15 | 6 | 3 | 0 | 0 | 1 | 1 | 3 | 1 | 0 |
| | Beerwort agar | 11 | 4 | 4 | 0 | 0 | 2 | 1 | 0 | 0 | 0 |
| X2 | Nutrient agar | 16 | 5 | 0 | 2 | 0 | 2 | 5 | 2 | 0 | 0 |
| | Yeast-glucose agar | 11 | 3 | 0 | 0 | 0 | 0 | 7 | 1 | 0 | 0 |
| | Beerwort agar‡ | — | — | — | — | — | — | — | — | — | — |
| D11 | Nutrient agar | 10 | 3 | 1 | 0 | 2 | 0 | 0 | 0 | 0 | 4 |
| | Yeast-glucose agar | 13 | 3 | 4 | 0 | 2 | 0 | 0 | 0 | 0 | 4 |
| | Beerwort agar | 15 | 2 | 2 | 0 | 2 | 0 | 2 | 0 | 0 | 7 |

* The figures for *Proteus vulgaris* do not give a representative picture of their numbers in the cheese, owing to their swarming properties.

† A single colony of *Oospora* was sometimes picked off a plate with more than one bacterial colony. The figures given for *Oospora*, therefore, do not necessarily represent the true proportions of the organisms in the cheese.

‡ Results not available.

### *Litmus milk reactions of cultures*

The reactions produced by the organisms in litmus milk are summarized in Table 12. In the examination of the slip-coat cheese samples, however, the numbers of colonies selected from the media may not give a truly representative picture of the flora owing to the swarming growth of *Proteus vulgaris* in samples DS1 and X2, and the formation of large mould colonies in sample D11. The table, therefore, gives an indication of the types of reactions which the organisms produce but does not necessarily indicate the relative proportions or the extent of the various changes.

Table 12. *Reactions in litmus milk by cultures isolated from surface scrapings*

| Litmus milk changes 14 days at 30° C. | No. of organisms DS1 | X2 | D11 |
|---|---|---|---|
| Total cultures examined ... | 37 | 27 | 38 |
| Acid and coagulation | 18 | 4 | 10 |
| Acid coagulation and digestion | 0 | 10 | 0 |
| Acid, no coagulation | 3 | 6 | 8 |
| Little or no change | 8 | 0 | 0 |
| Sweet clot | 0 | 2 | 0 |
| Alkalinity | 1 | 2 | 20 |
| Marked digestion | 7 | 3 | 0 |
| Slight digestion | 0 | 0 | 0 |

The table indicates that actively proteolytic and/or alkali-forming organisms, as well as acid-producing organisms, were present in the three samples of slip-coat cheese scrapings. Organisms producing an acid clot with digestion were limited to the slip-coat sample X2. Although the flora of the normal cheese samples V7 and X2 contained mainly acid-producing organisms, that of samples L1, L2 and L3 contained a high proportion of digesting and/or alkali-forming organisms. As the figures in the above table are limited, it is not possible to say that any change occurred to a lesser or greater extent in the slip-coat cheese samples than in the normal samples.

## DISCUSSION

The object of this investigation was to obtain information on the causes of slip-coat in Stilton cheese, with particular reference to the significance of micro-organisms in the development of the fault. The observations recorded provide some information on the conditions at the surface of Stilton cheese at the time of scraping.

During the course of the work an inquiry was made amongst a number of Stilton cheese manufacturers for information on the incidence of slip-coat formation and its cause and control. Definite evidence of conditions was difficult to obtain at some of the dairies. Moreover, few of the manufacturers made determinations of acidity, but relied entirely on the 'feel' of the curd to assess the stage of development and the suitability of the curd for moulding. Although the views and experiences of the manufacturers were found to be conflicting on certain points, some points of interest emerged from the inquiry.

It has been shown in this work that the surface of normal Stilton cheese contains very large numbers of micro-organisms at the time of scraping. The maximum total count obtained on yeast-glucose agar was 17,500 millions per gram.

As in Limburger and other varieties of soft cheese, the pH at the surface of Stilton cheese increases more rapidly than that of the interior. It is suggested that micro-organisms play a part in this increase in pH, but that the possible influence of the chemical composition of the surface of the cheese should not be overlooked. The pH's at the surface of different samples of normal Stilton cheese show a wide variation, and appear to influence the types of organisms there. The surface flora of normal Stilton cheese may vary from sample to sample. Yeasts, however, were invariably found at the time of scraping. Lactic acid bacteria also form part of the normal surface flora of Stilton cheese at scraping. Where the pH of the surface of the cheese is in the range 5·0–6·0, yeasts and lactic acid organisms predominate; the latter are usually lactobacilli, but, in young cheese, streptococci may be most numerous. Coliform organisms may also occur in small numbers, but, in the samples examined, their appearance was limited to the raw-milk cheese. Diphtheroid rods and pigmented cocci may also be present in small numbers. At higher pH's (>6·0) the composition of the flora is different. The numbers of yeasts and lactic acid organisms are lower, while diphtheroid rods and pigmented cocci are more numerous. Aerobic spore-forming bacteria may also occur. The count of moulds at the surface of the cheese is usually comparatively low at the time of scraping. The flora in the samples examined was restricted to species of *Penicillium*, *Mucor* and *Oospora*.

The results indicate that the surface flora of pasteurized milk cheese from different dairies may show wide variations. It appears, therefore, that pasteurization of milk for the manufacture of Stilton cheese does not determine the surface flora of the cheese at the scraping stage. However, correlation of the results of laboratory examinations with the nature of the coats formed on the cheese suggests that the types of micro-organisms present at the surface at the scraping stage determine the type of coat formed.

From the present findings it is not possible to draw any conclusions on the significance of *Proteus vulgaris* in the development of the initial stages of slip-coat formation in samples DS1 and X2. *Pr. vulgaris* is actively proteolytic. If proteolysis occurs at the surface of the cheese, it may be that the character of the cheese will be altered, and softening may result. It is obvious, however, that if the organisms are significant in the development of the fault, they must be able to grow and produce changes under the conditions prevailing at the surface of the cheese. Two of the factors determining their growth will be the pH and the salt content. The findings of Cornish & Williams (25) indicate that *Pr. vulgaris* can grow and produce changes within Stilton cheese. The question arises, however, as to the extent of growth which can occur in slip-coat samples at pH 5·8. In slip-coat samples at least 100 millions per gram of *Pr. vulgaris* were found. It has been established that the organism can grow on a nutrient agar medium at pH 5·5, but this does not establish its ability to grow and produce changes in cheese at the same pH. Changes are produced very slowly in litmus milk at pH 6·0.* On the evidence presented it seems unlikely, therefore, that *Pr. vulgaris* was sufficiently active at the pH (5·8) found in the samples to bring about the marked softening which occurred. Further, the pH of 5·8 suggests that the breakdown of casein was no more extensive in the slip-coat samples than in many of the normal samples. In cheese sample X2, *Pr. vulgaris* was accompanied by *Streptococcus liquefaciens* which is active at pH 5·8. *Str. liquefaciens* forms an acid clot and digests casein. It is possible, therefore, that this organism, in large numbers,

* Litmus milk adjusted to pH 6·0 with a five per cent solution of orthophosphoric acid.

attacks the casein slightly at the surface of the cheese. *Str. liquefaciens* may, therefore have been at least a contributory factor in the development of the slip-coat in sample X2.

*Oospora lactis*, found in large numbers in sample D11, is aciduric, and it can be assumed, therefore, that the organism could grow at the surface of the cheese from the time of manufacture. Although *O. lactis* grows slowly, it seems likely that the high pH (7·3) in sample D11 was due, partly, if not entirely, to the alkalinity produced by the organism.

The results obtained from the three samples suggest that while micro-organisms may be a contributory factor in the development of the initial stages of slip-coat, in some cheese they are not the only factor involved. It seems likely, however, that the micro-organisms found in the slip-coat samples will be more important in the development of the later stages of the fault, particularly when the moisture content is high.

It has been shown that an increase in pH occurs at the surface of normal Stilton cheese at the time when scraping is carried out. It can be assumed, therefore, that a similar increase in pH occurs at the surface of slip-coat cheese. The rate of growth of *Proteus vulgaris* increases rapidly with a rise in pH between 6·5 and 8·0. It was found that changes in litmus milk taking 5 days to develop at pH 7·0 were produced in 1 day at pH 8·0.* It is therefore probable that provided other conditions are favourable, *Pr. vulgaris* will develop rapidly as the pH of the surface of the cheese increases.

The moisture content at the surface of the cheese will have a considerable effect on growth. Consequently cheese in which drainage has been slow, either during manufacture or in the early stages of storage, are more likely to promote the growth of *Pr. vulgaris* than those in which normal drainage has taken place. Growth of *Pr. vulgaris* in cheese is likely to give rise to changes similar to those which occur in milk. Such changes in the casein can be expected to alter the character of the cheese. It seems probable, therefore, that the advanced stages of slip-coat are due, in part at least, to the growth of such organisms as *Pr. vulgaris*.

It is not possible from the results to form any conclusions on the direct effects of moisture in the development of slip-coat. The findings of Bryant & Hammer (16) on the effect of moisture in the development of the soft-edge defect, the practical views of early workers on slip-coat formation, and the evidence obtained in this work indicate that further information is required on the influence of moisture on slip-coat development. The problem of moisture content in the cheese is linked with that of drainage, which, in turn, is influenced by the storage conditions.

Observations at the Stilton dairies have failed to explain why one or two isolated cheese develop slip-coat while the surrounding ones do not. The growth of micro-organisms in some samples of cheese is a possible explanation, but the available evidence does not support it. The possibilities of slightly different temperatures and humidities have been considered but no conclusive evidence has been obtained. It is clear, however, that whereas slip-coat does not necessarily develop in a high humidity and temperature, it is more likely to occur under such conditions.

It has been established that slip-coat occurs more frequently in cheese made from raw than in those made from pasteurized milk. Here there are two factors which may be considered: the destruction by pasteurization of many organisms in the raw milk, and differences in the drainage of raw and pasteurized milk cheese. At several of the Stilton cheese-making dairies the milk supplies are obtained from farms where production

* Milk adjusted to pH 8·0 by means of a 20% solution of dipotassium hydrogen phosphate.

methods are poor and where water supplies are inadequate for milk production. Bacteriological examination of some of the raw-milk supplies has shown that the bacterial counts are high, and the flora varied. Where, however, H.T.S.T. pasteurization of the milk is carried out, the numbers of bacteria are considerably lower and the flora is confined to heat-resistant organisms and to contaminants which gain access to the milk after heating. Pasteurization of the milk was carried out on the spot at two of the dairies concerned and high standards were maintained, so that the numbers of contaminants were low. It is unlikely, therefore, that bacteria such as *Pr. vulgaris* will be present in large numbers in pasteurized milk cheese. The chances of faults of bacterial origin developing are much less in pasteurized milk cheese than in raw-milk cheese. The results of the laboratory examination of slip-coat cheese suggest, however, that bacteria are of little, if any, significance in the development of the initial stages of slip-coat. In considering the lower incidence of slip-coat in pasteurized milk cheese it is difficult, therefore, to attach much significance to the direct effect of pasteurization of the flora of the milk. Conflicting factors are present in considering the differences in drainage between pasteurized and raw-milk cheese. Some workers claim that drainage from the cheese is slower in pasteurized milk cheese because of the greater retentive capacity of the soluble milk proteins after heat treatment. Against this, however, the dairies using pasteurized milk find that the addition of only minute quantities of starter is necessary to obtain acid development and the right degree of drainage in the curd before moulding. It is difficult, therefore, to be sure whether or not better drainage occurs in pasteurized milk cheese. One important factor with pasteurized milk, however, is that a greater degree of control in manufacture can be maintained. In raw-milk cheese, with or without starter, the course of acid production may be affected by the miscellaneous flora of organisms present. This irregularity is overcome in pasteurized milk in which, with an active starter, acid production can be controlled from day to day. Consequently the rate of drainage from the curd can also be controlled. It is possible, therefore, that the better control of manufacture, which is possible when pasteurized milk is used, accounts for the lower incidence of slip-coat in pasteurized milk cheese.

A common opinion amongst the manufacturers was that curd in which a high acidity is allowed to develop before moulding is more prone to slip-coat formation. This factor was regarded by four of the manufacturers as the most important cause of the fault. Conditions of high acidity in the curd prior to moulding are more likely to develop in summer at dairies where there is no temperature control, and where raw milk, with or without the addition of starter, is used. By the time the curd is moulded about 22 hr. after renneting a titratable acidity of 2·0% may have developed. Without further evidence it is difficult to reconcile the manufacturer's views on the influence of a high acidity in the development of slip-coat with the results obtained from the laboratory examination of samples. Where high acidities are used at moulding more drainage from the curd will have occurred. A high moisture content is therefore less likely to be present in cheese of high acidity. If a high moisture content influences slip-coat, it can be assumed that the effect of a high acidity would be to reduce the chance of slip-coat development. High acidities would also effect a greater control over the flora of the cheese for a short time. The manufacturers' opinion on the influence of high acidity in slip-coat development is that the cheese is ready for scraping earlier than normal cheese, and that if the scraping is delayed, considerable softening may occur at the surface. It

seems possible that this quicker maturity will, in fact, occur in high-acidity cheese, yet it is difficult to connect the possible causes of softening at the surface of the cheese with a high acidity.

## REFERENCES


(1) Percival, J. & Mason, G. H. (1913). *J. agric. Sci.* **5**, 223.
(2) Hiscox, E. R., Harrison, J. & Wolf, J. Z. (1940). *Proc. Soc. agric. Bact.* p. 23.
(3) Davis, J. G. (1932). *J. Dairy Res.* **3**, 241.
(4) Laxa, O. (1899). *Zbl. Bakt.* II. Abt. 5. Cited by Yale (1943), *Bull. N.Y. St. agric. Exp. Sta.* no. 268.
(5) Wolff, A. (1909). *Milchw. Zbl.* **5**, 414.
(6) Wolff, A. (1910). *Zbl. Bakt.* II. Abt. 28, 417.
(7) Steinfatt, F. (1930). *Milchw. Forsch.* **9**, 1.
(8) Kelly, C. D. (1937). *J. Dairy Sci.* **20**, 239.
(9) Kelly, C. D. & Marquardt, J. C. (1939). *J. Dairy Sci.* **22**, 309.
(10) Macy, H. & Erekson, J. A. (1937). *J. Dairy Sci.* **20**, 464.
(11) Macy, H. &. Erekson, J. A. (1937). Unpublished data cited by Kelly & Marquardt (1939), *J. Dairy Sci.* **22**, 309.
(12) Yale, M. W. (1943). *Res. Bull. N.Y. St. agric. Exp. Sta.* no. 283.
(13) Sutherland Thomson, G. (1925). *Butter and Cheese.* London: Lockwood and Son.
(14) Lord, L. J. (1925). *Practical Butter and Cheesemaking.* London: Benn.
(15) Ministry of Agriculture & Fisheries (1938). *Bull.* 43.
(16) Bryant, H. W. & Hammer, B. W. (1940). *Ia. Agric. Exp. Sta. Res. Bull.* 283.
(17) Harrison, J. (1938). *Proc. Soc. agric. Bact.* p. 12.
(18) British Standards Institution (1938). Publication no. 770.
(19) Mattick, E. C. V. (1938). *J. Dairy Res.* **9**, 233.
(20) Hunter, G. J. E. (1950). *J. Dairy Res.* **17**, 79.
(21) Slatter, W. L. & Halvorson, H. O. (1947). *J. Dairy Sci.* **30**, 231.
(22) Brindley, M. (1948). Unpublished data.
(23) Davis, J. G. (1935). *J. Dairy Res.* **6**, 175.
(24) *Bergey's Manual of Determinative Bacteriology*, 6th ed. (1948). Edited by Breed, R. S., Murray, E. G. D. & Hitchens, A. P. London: Ballière, Tindall and Cox.
(25) Cornish, E. C. V. & Williams, R. S. (1917). *Biochem. J.* **11**, 181.


(*MS. received for publication* 1 *April* 1953)

637.333.3:576.8.637.361.2

# 543. METHODS FOR THE GROWTH AND ENUMERATION OF ANAEROBIC SPORE-FORMERS FROM CHEESE, WITH OBSERVATIONS ON THE EFFECT OF NISIN

By A. HIRSCH and ELISABETH GRINSTED
*National Institute for Research in Dairying, University of Reading*

(With 2 Figures)

## INTRODUCTION

Severe losses are caused in some types of cheese by the growth of spore-forming anaerobes. The species most commonly involved is *Clostridium butyricum* and its variants, but other clostridia such as *Cl. sporogenes* (*Bacillus putrificus* of Dorner (1)) may also be concerned. Existing methods for the study of the properties of these organisms are inadequate, a fact on which Rosenberger (2) has commented. This work has been particularly concerned with devising a medium suitable for all clostridia, which is clear, simple to prepare, and which requires no special anaerobic precautions in its use.

Antibiotics offer the prospect of inhibiting the gas formation, though possibly not the growth, of the sporing anaerobes. It has, in fact, been shown (3,4) that nisin itself, or starters containing nisin-producing streptococci, inhibit the gas production of these organisms in cheese. Subtilin and other antibiotics when used in canning have also given some encouraging results (5,6,7). This paper, although the work is not complete, describes some further effects of nisin on the anaerobes of importance to the cheese-making industry.

## EXPERIMENTAL

### *Cultures of clostridia*

The anaerobic spore-formers used in this work came from a variety of sources. Some were obtained from the National Collection of Type Cultures, and one was isolated by Dr O. Richard from blown cheese in Zürich. Most of the strains were isolated from samples of cheese from Sweden, Denmark and France. Some strains were also isolated from processed cheese manufactured in this country.

As several authors point out (e.g. van Beynum & Pette (8)), absolute purity of the strains may be difficult to achieve but is essential. For purifying we used both the Burri technique (Burri (9), Hall (10)) and a modified method of streaking on agar plates for picking individual colonies. This latter method, although it requires anaerobic jars, is convenient and yields colonies which probably originate from a single cell. Fig. 1 is a diagrammatic representation of this modification. Tenfold dilutions of the culture were prepared, and from each dilution tube five separate loopfuls were placed on dried 2% agar medium. The dilutions were chosen so as to yield at least one row where, say, only two of the five loopfuls contained living cells. In the next dilution, fewer or none of the loopfuls should give growth. It may be then assumed that the culture has been diluted sufficiently to give one viable organism per loopful. Although motile organisms were frequently encountered, they did not cause real difficulty, and a medium containing

6–8% agar, as recommended by Hayward & Miles (11), to reduce the motility of *Proteus* cultures was not found to be necessary. We have, however, observed that for the ready production of colonies aged cultures were more suitable than young cultures, an observation confirming earlier work of McClung (12). The appearance of the colonies was variable from plate to plate.

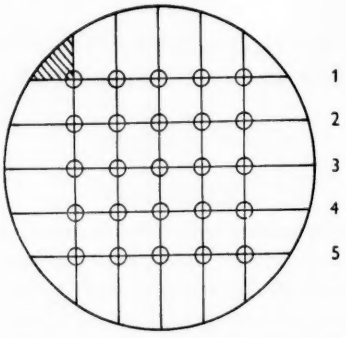



Fig. 1. Diagram of plate for single-colony isolation. Nos. 1–5 indicate 10-fold dilution of culture, dilutions used depending on the numbers of organisms per ml. of culture.

After a freshly isolated culture had been purified by 4–5 repeated colony pickings, its identity could be established by following the key of Spray (13) or Prévot (14). Most of our strains appeared to be *Clostridium sporogenes*; many strains fermented lactate and also liquified gelatin and we did not encounter *Cl. tyrobutyricum* of van Beynum & Pette (8). A random selection of thirteen strains isolated from cheese was injected intramuscularly into guinea-pigs. Only one strain appeared to be pathogenic and this resembled *Cl. histolyticum*.

### *Media*

For this work we required a clear liquid medium which would support rapid growth and good gas production without special anaerobic precautions. These requirements were satisfied by a medium based on yeast-glucose broth which was in general use in these laboratories. It was supplemented with 0·5% sodium acetate (Bhat & Barker (15)), with 0·1% soluble starch (Olsen & Scott (16)), with 0·05% agar (Spray (13)), and in addition it was found necessary to reduce the oxidation-reduction potential.

The results of some eH measurements on a basic medium containing various reducing substances are given in Table 1. Iron wire and the inorganic salts tested were good reducing agents. Thioglycollate, as previously used in Brewer's medium (17), was also very effective. Its use was, however, criticized by Clark (18), who, in extensive trials with thermophilic anaerobes, found it to be toxic. Table 2 gives the results of growth experiments using sodium thioglycollate. It can be seen that this compound is toxic not only to the thermophilic anaerobes but also to *Cl. butyricum*, *sporogenes* and *welchii*. The effect is less marked with *Cl. histolyticum*. The reduction in eH could not alone be responsible, since good growth was obtained with the lower glycollate concentrations after overnight incubation.

It was decided that cysteine best met the requirements as a reducing agent and a concentration of 0·05% was used.

The medium finally selected, which for convenience was called the Reinforced Clostridial Medium (R.C.M.), had the following composition: Yeast extract (Yeastrel) 0·3%, peptone (Evans) 1%, meat extract (Lab-lemco) 1%, glucose 0·5%, sodium acetate 0·5%, cysteine 0·05%, soluble starch 0·1% and agar 0·05%. For solid media the agar content was increased to 2%.

Table 1. *The* eH *of certain media*

| Composition* | eH, after heating to 100° C. and cooling |
|---|---|
| Robertson's cooked meat medium | + 40 |
| Basic medium | +238 |
| +0·04% glucose | +279 |
| +0·9% glucose | +246 |
| +0·2% sodium sulphite | +208 |
| +0·1% sodium sulphide | +138 |
| +0·02% cysteine | +204 |
| +0·05% cysteine | +164 |
| +0·1% cysteine | +132 |
| +iron wire | +144 |
| +0·1% sodium thioglycollate | + 72 |

* The basic medium contained 1% peptone, 0·5% Yeastrel, 0·1% glucose, 0·1% starch, pH to 7·6.

Table 2. *The effect of sodium thioglycollate on the growth of some clostridia*

| | Overnight growth from small inocula of | | | |
|---|---|---|---|---|
| | *Cl. butyricum*, N.C.T.C. 7423 | *Cl. sporogenes*, Cl. 6 | *Cl. welchii*, N.C.T.C. 8503 | *Cl. histolyticum*, Cl. 9 |
| Sodium thioglycollate: Batch 1: 0·1% | − | − | − | + |
| 0·01% | + + + | + + + | + + + | + + + |
| Batch 2: 0·1% | − | − | − | + |
| 0·01% | + + + | + + + | + + + | + + + |
| Batch 3: 0·1% | − | − | + | + |
| 0·01% | + + + | + + + | + + + | + + + |

Although R.C.M. was designed primarily for *Cl. butyricum* and a subculture of this organism gave visible growth after 2–3 hr. incubation, it also proved to be a suitable medium for other clostridia. R.C.M. was compared with other well-established media by observing the rate of growth and gas production from increasing dilutions of inocula. In Table 3 a comparison is made between R.C.M. and the Yessair medium (Wynne & Foster (19)) which was used with and without a yeast-extract supplement, a meat-extract-liver medium (V.F.), especially recommended by the French workers Weinberg, Nativelle & Prévot (20), the corn-liver medium of McClung & McCoy (21) and the peptone-supplemented milk medium recommended by Crossley (22). It is evident that as an all-round medium, R.C.M. was better than any of the others tried, Yessair's medium being second best. The corn-liver mash, V.F. and Crossley milk media were inferior both as regards the rate of growth, gas production, growth from small inocula and ease of observation.

No special precautions are needed for anaerobic cultivation in R.C.M. beyond the usual boiling for ½ hr. when the medium has been exposed to air for some time. However, with small inocula of spore suspensions, which may take 2–4 days to germinate, there is a danger of the medium becoming oxygenated, and the effect of a seal was observed in a quantitative experiment with counts. It was then found that a significantly higher

count was obtained when seals were used. Because a dilution method of counting was preferred, it was necessary for the seal to float on the surface of the broth. Paraffin wax which is usually used shrinks away from the glass, while agar seals may not float on liquid media. The seal which was eventually devised is a mixture of 'Vaseline' and agar emulsified with Tween 80 and consists of 'Vaseline' 50 g., agar 2 g., Tween 80 1 ml. and distilled water 50 ml. The ingredients are measured out, dissolved in the steamer, distributed in 50 ml. quantities and sterilized by autoclaving. After the seal has set it

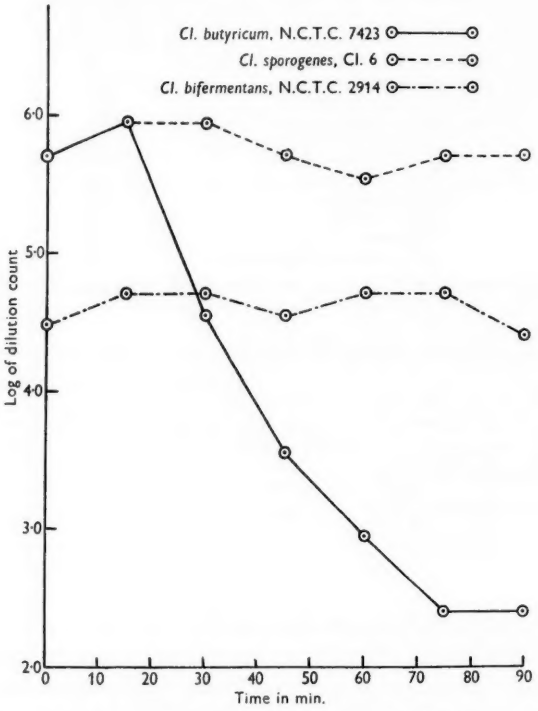



Fig. 2. The effect of the culture fluid of a nisin-producing *Streptococcus* (no. 12), grown in milk, on spores of *Clostridia*.

may be remelted by placing the bottles in a boiling water-bath. When poured on top of a liquid medium a $\frac{1}{2}$ cm. thick layer sets sufficiently firmly to permit inversion of the tubes.

A disadvantage of the use of R.C.M. is that organisms tend to clump in it and for counting purposes extra care is necessary. Pepsin, bile salts, sodium oleate, Lubrol W, Saponin, Tween 80, and the enzyme preparation 'Bacterase' were added in an attempt to prevent this clumping, but all were unsuccessful. Further, R.C.M. is not a selective media for clostridia. Streptococci and lactobacilli in particular flourish in it, but if the glucose is replaced by 0·5% sodium lactate the growth of the lactic organisms is much reduced. Most of the anaerobes tested grow and produce gas well in media containing lactate.

The antibiotic aerosporin at a concentration of 15–20 $\mu$g./ml. of the medium was found

Table 3. *Comparison of media for growth of clostridia*

Medium

Table 3. *Comparison of media for growth of clostridia*

| Inoculum | Dilution | Medium: R.C.M. Hours 18 | R.C.M. Hours 24 | R.C.M. Days 2 | R.C.M. Days 3 | R.C.M. Days 5 | Yessair's medium Hours 18 | Yessair's medium Hours 24 | Yessair's medium Days 2 | Yessair's medium Days 3 | Yessair's medium Days 5 | Yessair's medium + 0·3% yeast extract Hours 18 | Yessair's medium + 0·3% yeast extract Hours 24 | Yessair's medium + 0·3% yeast extract Days 2 | Yessair's medium + 0·3% yeast extract Days 3 | Yessair's medium + 0·3% yeast extract Days 5 | Corn-liver mash Hours 18 | Corn-liver mash Hours 24 | Corn-liver mash Days 2 | Corn-liver mash Days 3 | Corn-liver mash Days 5 | Medium v.F. Hours 18 | Medium v.F. Hours 24 | Medium v.F. Days 2 | Medium v.F. Days 3 | Medium v.F. Days 5 | Crossley's B.C.P. milk Hours 18 | Crossley's B.C.P. milk Hours 24 | Crossley's B.C.P. milk Days 2 | Crossley's B.C.P. milk Days 3 | Crossley's B.C.P. milk Days 5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Cl. butyricum*, N.C.T.C. 7423 | $10^{-3}$ | 5 | 5 | 5 | 5 | 5 | 4 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | No visible growth* | | | | | No growth | | | | | 0 | 4 | 4 | 4 | 4 |
| | $10^{-4}$ | 5 | 5 | 5 | 5 | 5 | 2 | 4 | 4 | 4 | 4 | 5 | 5 | 5 | 5 | 5 | | | | | | | | | | | 0 | 0 | 0 | 0 | 0 |
| | $10^{-5}$ | 0 | 1 | 3 | 3 | 3 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | | | | | | | | | | | 0 | 0 | 0 | 0 | 0 |
| | $10^{-6}$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | | | | | 0 | 0 | 0 | 0 | 0 |
| | $10^{-7}$ | 1 | 2 | 2 | 2 | 2 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | | | | | | | | | | | 0 | 0 | 0 | 0 | 0 |
| *Cl. tyrobutyricum*, 4A/M | $10^{-3}$ | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 1 | 1 | 1 | 1 | 1 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| | $10^{-4}$ | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 0 | 0 | 0 | 0 | 0 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| | $10^{-5}$ | 5 | 5 | 5 | 5 | 5 | 4 | 4 | 4 | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 4 | 4 | 4 | 4 | 1 | 1 | 1 | 1 | 1 |
| | $10^{-6}$ | 5 | 5 | 5 | 5 | 5 | 2 | 4 | 4 | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 2 | 2 | 2 | 1 | 3 | 3 | 3 | 3 |
| | $10^{-7}$ | 4 | 4 | 4 | 4 | 4 | 2 | 2 | 2 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 2 | 2 | 2 | 0 | 0 | 0 | 0 | 0 |
| *Cl. bifermentans*, N.C.T.C. 2914 | $10^{-3}$ | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | No visible growth* | | | | | 5 | 5 | 5 | 5 | 5 | 0 | 0 | 5 | 5 | 5 |
| | $10^{-4}$ | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | | | | | | 4 | 5 | 5 | 5 | 5 | 0 | 0 | 5 | 5 | 5 |
| | $10^{-5}$ | 0 | 5 | 5 | 5 | 5 | 0 | 5 | 5 | 5 | 5 | 0 | 5 | 5 | 5 | 5 | | | | | | 0 | 0 | 5 | 5 | 5 | 0 | 0 | 5 | 5 | 5 |
| | $10^{-6}$ | 0 | 1 | 5 | 5 | 5 | 0 | 0 | 5 | 5 | 5 | 0 | 0 | 5 | 5 | 5 | | | | | | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 2 | 2 |
| | $10^{-7}$ | 0 | 0 | 5 | 5 | 5 | 0 | 0 | 5 | 5 | 5 | 0 | 0 | 5 | 5 | 5 | | | | | | 0 | 3 | 5 | 5 | 5 | 0 | 0 | 5 | 5 | 5 |
| *Cl. welchii* (?) | $10^{-3}$ | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | | | | | | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| | $10^{-4}$ | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | | | | | | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| | $10^{-5}$ | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | | | | | | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| | $10^{-6}$ | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | | | | | | 5 | 5 | 5 | 5 | 5 | 4 | 5 | 5 | 5 | 5 |
| | $10^{-7}$ | 5 | 5 | 5 | 5 | 5 | 1 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | | | | | | 2 | 5 | 5 | 5 | 5 | 2 | 5 | 5 | 5 | 5 |
| *Cl. sporogenes*, N.C.T.C. 532 | $10^{-3}$ | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | No visible growth* | | | | | 1 | 4 | 4 | 5 | 5 | 0 | 0 | 5 | 5 | 5 |
| | $10^{-4}$ | 5 | 5 | 5 | 5 | 5 | 1 | 4 | 5 | 5 | 5 | 3 | 5 | 5 | 5 | 5 | | | | | | 0 | 2 | 4 | 5 | 5 | 0 | 0 | 5 | 5 | 5 |
| | $10^{-5}$ | 0 | 1 | 5 | 5 | 5 | 0 | 1 | 5 | 5 | 5 | 0 | 4 | 5 | 5 | 5 | | | | | | 0 | 0 | 4 | 4 | 4 | 0 | 0 | 5 | 5 | 5 |
| | $10^{-6}$ | 0 | 0 | 4 | 5 | 5 | 0 | 0 | 4 | 5 | 5 | 0 | 1 | 4 | 5 | 5 | | | | | | 0 | 0 | 2 | 2 | 2 | 0 | 0 | 0 | 0 | 0 |
| | $10^{-7}$ | 0 | 0 | 4 | 5 | 5 | 0 | 0 | 3 | 4 | 4 | 0 | 0 | 4 | 5 | 5 | | | | | | 0 | 0 | 2 | 2 | 2 | 0 | 0 | 0 | 0 | 0 |
| *Cl. septicum* | $10^{-3}$ | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | No visible growth* | | | | | 0 | 0 | 1 | 1 | 1 | | No growth | | | |
| | $10^{-4}$ | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | | | | | | 0 | 0 | 0 | 1 | 1 | | | | | |
| | $10^{-5}$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | 0 | 0 | 0 | 0 | 0 | | | | | |
| | $10^{-6}$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | 0 | 0 | 0 | 0 | 0 | | | | | |
| | $10^{-7}$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | 0 | 0 | 0 | 0 | 0 | | | | | |
| *Cl. oedematiens* | $10^{-3}$ | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 4 | 5 | 5 | 5 | 5 | No visible growth* | | | | | No growth | | | | | No growth | | | | |
| | $10^{-4}$ | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 4 | 5 | 5 | 5 | 5 | | | | | | | | | | | | | | | |
| | $10^{-5}$ | 5 | 5 | 5 | 5 | 5 | 1 | 2 | 2 | 2 | 2 | 0 | 1 | 1 | 1 | 1 | | | | | | | | | | | | | | | |
| | $10^{-6}$ | 1 | 3 | 4 | 4 | 4 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | | | | | | | | | | | | | | | |
| | $10^{-7}$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | | | | | | | | | |

Five tubes were set up for each dilution. The figures in the table indicate the number of tubes which show active growth
N.B. In Crossley's milk medium 0·03% B.C.P. was used instead of 0·01% as specified.
* Media opaque; growth if present not visible.

not to inhibit clostridia but was markedly inhibitory to contaminating Gram-negative organisms (23). The aerosporin solution was made up and sterilized separately and added to R.C.M. as required.

*Counting methods*

Although many workers in this field have relied for counts on deep plates, e.g. Wynne & Foster (19), Andersen (24), we found the anaerobes isolated from cheese to be such vigorous gas producers that when some 100–300 colonies were present on a plate and the colonies had grown to a reasonable size, the agar was frequently so badly torn as to make the plates unusable. The method of Miles & Misra (25) was then tried. In this method those

Table 4. *Comparison between the Miles & Misra and dilution methods of counting of a strain of* Clostridium sporogenes (Cl. 6)

| | Dilution count | | Miles & Misra count | |
|---|---|---|---|---|
| | Before heating* | After heating | Before heating | After heating |
| Test 1 | 35,000,000 | 5,000,000 | 4,300,000 | 1,400 |
| Test 2 | 5,000,000 | 800,000 | 1,500,000 | 5,280 |

* Heating was to 80° C. for 10 min.
The culture was grown in R.C.M. and counted in R.C.M. broth and agar.

colonies are counted which develop from a standard drop placed on the surface of the agar. Either special plates or anaerobic jars are required for this technique. Another counting method which has been used frequently with these organisms is the dilution method, e.g. McClung & McCoy (21). Typical results of experiments comparing the Miles & Misra and dilution methods are given in Table 4. The Miles & Misra technique gave counts of about one-tenth of those obtained by the dilution method when vegetative cells and spores were being counted. With spore suspensions the discrepancy was increased further, the dilution count yielding answers which were at times about 10,000 times higher than the Miles & Misra counts. The dilution method had two further important advantages: the day-to-day variation in count was noticeably smaller than with the Miles & Misra method, and it was easier to perform.

*Sporulation*

Ingram & Robinson (26), using digest agar or broth incubated for 2–4 days, were able to produce a suspension of *Cl. botulinum* which consisted almost entirely of vegetative cells. With the R.C.M. this was not possible; overnight cultures usually contained a significant number of spores. Young cultures, 4–8 hr. old, had to be used for work involving vegetative cells only. The presence of calcium or oxalate, shown by Bordet & Renaux (27) to have a profound effect on the sporulation of *Bacillus anthracis*, had no effect on that of several strains of anaerobes which were tested. Certain saturated fatty acids have also been shown to have marked anti-sporulation activity with aerobic spore-formers (28), but although capric acid had some effect this was not consistent. *Clostridium welchii* failed to produce spores in R.C.M.

*The effect of nisin on some clostridia*

Strains of *Cl. sporogenes*, *butyricum* and *bifermentans* were used throughout this phase of the work. Very young cultures were used to investigate the effect of nisin on cells, 5-day cultures were used for a mixed culture of vegetative cells and spores, and aged

cultures, 2–3 weeks old, were used for the preparation of spore suspensions. These spore suspensions were prepared by centrifuging the culture, taking the precipitate up in one-third volume of saline, shaking with glass beads and finally heating to 80° C. for 10 min. The count of each suspension was determined so that for assay the same approximate weight of inoculum could be used.

Instead of isolated nisin, the culture fluid from one of the nisin-producing streptococci (strain 12 of Hirsch & Grinsted (29)) was used. This was chosen because it had already been shown that strain 12 inhibited the development of gas-producing anaerobes when used as a starter in cheese-making (4). The streptococci were grown overnight in litmus milk, the clotted milk was brought to the boil in a water-bath and the clear whey serially diluted in R.C.M. which was then inoculated with a suitable dilution of the test organism. All assays and counts were made at least in duplicate.

Table 5. *The effect of nisin on cultures composed of spores and cells*

| | Inoculum cells/ml. final concentration | Growth control | | Dilutions of strain 12 culture fluid 1/10 | | 1/20 | | 1/40 | |
|---|---|---|---|---|---|---|---|---|---|
| | | Growth | Gas | Growth | Gas | Growth | Gas | Growth | Gas |
| *Cl. butyricum*, N.C.T.C. 7423 | $2 \times 10^6$ | + + + | + + + | + + + | + | + + + | + | + + + | + + + |
| | $2 \times 10^4$ | + + + | + + + | − | − | − | − | + + + | + |
| *Cl. sporogenes*, Cl. 6 | $11 \times 10^6$ | + + + | + + + | + + + | + + + | + + + | + + + | + + + | + + + |
| | $11 \times 10^4$ | + + + | + + + | + | − | + + + | + + + | + + + | + + + |
| *Cl. bifermentans*, N.C.T.C. 2914 | $5 \times 10^6$ | + + + | + + + | + + + | ± | + + + | + + + | + + + | + + + |
| | $5 \times 10^4$ | + + + | + + + | + + + | + + + | + + + | + + + | + + + | + + + |

The positives and negatives indicate extent of growth or no growth of the test organism.

Table 5 gives some results of the effect of nisin on mixtures of cells and spores. On the whole, none of the strains tested was susceptible. The results of these experiments were often contradictory. The effect of nisin on young cells is shown in Table 6, and it can be seen that, in contrast to the older cells, the younger were very susceptible. Table 7 shows the effect of nisin on spores.

Comparisons between the results of Tables 5, 6 and 7 are not easy because of the very different numbers of cells used in the different experiments, and Table 8 has been condensed from Tables 5, 6 and 7 for a comparison of the susceptibility of the spores, young cells and the spore/cell mixture of the three organisms. It can be seen from Table 8 that the three organisms behaved very differently. *Cl. butyricum* shows a slight sensitivity to nisin when a mixture of cells and spores is tested, but surprisingly the spores appear to be more sensitive than the cells. Thus the suspension of young cells was not inhibited at all, whereas the spore suspension was inhibited to 1/80. With *Cl. sporogenes* and *bifermentans* the cells and spores appear to be more nisin resistant, although if the inoculum is small, high titres of up to 1/640 can be obtained with both these organisms (Table 7).

Other experiments were carried out to determine whether nisin inhibits spore germination, or whether it is actively sporicidal. Preliminary tests were made by inoculating the dilution assay tubes into large volumes of media (*c.* 1:100), therefore diluting out the nisin present. None of the tubes showed any growth, but it was possible for the spores to be alive but unable to germinate because of the nisin adsorbed on them. Further experiments were therefore made using a heavier inoculum of spores, the nisin concentration being the same as in the dilution assays. The diluting-out technique was not used,

Table 6. *The effect of nisin on young cells of clostridia*

| | Inoculum cells/ml. final concentration | Growth control | | Nisin—dilutions of strain 12 culture fluid | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1/10 | | 1/20 | | 1/40 | | 1/80 | | 1/160 | | 1/320 | |
| | | Growth | Gas | Growth | Gas | Growth | Gas | Growth | Gas | Growth | Gas | Growth | Gas | Growth | Gas |
| *Cl. butyricum*, N.C.T.C. 7423 | 17,000 | + + + | + + + | + + + | + + + | + + + | + + + | + + + | + + + | + + + | + + + | + + + | + + + | + + + | + + + |
| | 170 | + + + | + + + | − | − | − | − | + | − | + + + | − | + + + | ± | + + + | + + + |
| | 1·7 | + + + | + + + | − | − | − | − | − | − | − | − | − | − | + + | ± |
| *Cl. sporogenes*, Cl. 6 | 18,000,000 | + + + | + + + | + + | ± | + + + | + + + | + + + | + + + | + + + | + + + | + + + | + + + | + + + | + + + |
| | 180,000 | + + + | + + + | − | − | − | − | + + + | + + + | + + + | + + + | + + + | + + + | + + + | + + + |
| | 1,800 | + + + | + + + | − | − | − | − | − | − | + | − | + + + | + + + | + + + | + + + |
| *Cl. bifermentans*, N.C.T.C. 2914 | 130,000 | + + + | + | + | − | + | − | + + + | + | + + + | + | + + + | + | + + + | + |
| | 1,300 | + + + | + | − | − | − | − | + + + | + | + + + | + | + + + | + | + + + | + |
| | 13 | + + + | + | − | − | − | − | − | − | + + | + | + + + | + | + + + | + |

The positives and negatives indicate extent of growth or no growth of the test organism.

Table 7. *The effect of nisin on spores of clostridia*

| | Inoculum spores/ml. final concentration | Growth control | | Nisin—dilutions of strain 12 culture fluid | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1/10 | | 1/20 | | 1/40 | | 1/80 | | 1/160 | | 1/320 | | 1/640 | | 1/1280 | |
| | | Growth | Gas | Growth | Gas | Growth | Gas | Growth | Gas | Growth | Gas | Growth | Gas | Growth | Gas | Growth | Gas | Growth | Gas |
| *Cl. butyricum*, N.C.T.C. 7423 | 3,500 | + + + | + + + | − | − | − | − | − | − | − | − | + + + | + + + | + + + | + + + | | | | |
| | 35 | + + + | + + + | − | − | − | − | − | − | − | − | − | − | − | − | + + + | + + + | + + + | + + + |
| *Cl. sporogenes*, Cl. 6 | 800 | + + + | + + + | − | − | − | − | − | − | + + + | + + + | + + + | + + + | + + + | + + + | | | | |
| | 8 | + + + | + + + | − | − | − | − | − | − | − | − | − | − | − | − | + + + | + + + | + + + | + + + |
| *Cl. bifermentans*, N.C.T.C. 2914 | 800 | + + + | + + + | − | − | − | − | − | − | + + + | + + + | + + + | + + + | + + + | + + + | | | | |
| | 8 | + + + | + + + | − | − | − | − | − | − | − | − | − | − | − | − | − | − | + + + | + + |

The positives and negatives indicate extent of growth or no growth of the test organism.

but samples for counting were taken at intervals of time. These results are given in Fig. 2, and it may be seen that nisin was actively sporicidal in the case of *Cl. butyricum* but that the count did not change in the cases of *Cl. sporogenes* and *bifermentans*. It appears that this antibiotic can act, according to the nature of the test organism and the numbers present, either sporicidally or as an inhibitor of germination.

Table 8. *The effect of nisin on clostridia*

| | | Inoculum (final concentration) | Nisin—dilutions of strain 12 culture fluid | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1/10 | 1/20 | 1/40 | 1/80 | 1/160 | 1/320 |
| *Cl. butyricum* N.C.T.C. 7423 | Spores and cells | 20,000 | − | − | + + + | | | |
| | Cells only | 17,000 | + + + | + + + | + + + | | | |
| | Spores only | 3,500 | − | − | − | − | + + + | + + + |
| *Cl. sporogenes* Cl. 6 | Spores and cells | 110,000 | + | + + + | | | | |
| | Cells only | 180,000 | − | − | + + + | + + + | | |
| | Spores only | 800 | − | − | − | + + + | | |
| *Cl. bifermentans*, N.C.T.C. 2914 | Spores and cells | 50,000 | + + + | | | | | |
| | Cells only | 130,000 | + | + | + + + | | | |
| | Spores only | 800 | − | − | − | + + + | + + + | |

The positives and negatives indicate extent of growth or no growth of the test organism.

Tables 5–7 show that gas production was inhibited more readily than growth, so it is possible that under certain circumstances clostridia could grow in cheese without obviously spoiling it through excessive gas production.

We wish to thank Dr M. Ingram for the data in Table 1 and Dr M. K. Stevens for those in Table 3.

## REFERENCES

(1) DORNER, W. (1938). *Lait*, **18**, 449.
(2) ROSENBERGER, R. F. (1951). *Proc. Soc. appl. Bact.* **14**, 161.
(3) MCCLINTOCK, M., SERRES, L., MARZOLF, J. J., HIRSCH, A. & MOCQUOT, G. (1952). *J. Dairy Res.* **19**, 187.
(4) HIRSCH, A., GRINSTED, E., CHAPMAN, H. R. & MATTICK, A. T. R. (1951). *J. Dairy Res.* **18**, 205.
(5) ANDERSEN, A. A. (1952). *J. Bact.* **64**, 145.
(6) CAMERON, E. J. & BOHRER, C. W. (1951). *Food Technol.* **5**, 340.
(7) WILLIAMS, O. B. & FLEMING, T. C. (1952). *Antibiotics and Chemotherapy*, **2**, 75.
(8) VAN BEYNUM, J. & PETTE, J. W. (1935/6). *Zbl. Bakt.* II. Abt. **93**, 198.
(9) BURRI, R. (1902). *Zbl. Bakt.* II, **8**, 533.
(10) HALL, I. C. (1920). *J. infect. Dis.* **27**, 576.
(11) HAYWARD, N. J. & MILES, A. A. (1943). *Lancet* ii, 116.
(12) MCCLUNG, L. S. (1935). *J. Bact.* **29**, 173.
(13) SPRAY, R. S. (1936). *J. Bact.* **32**, 135.
(14) PRÉVOT, A. R. (1948). *Manuel de classification et de détermination des bactéries anaérobies*, 2nd ed. Paris: Masson et Cie.
(15) BHAT, J. V. & BARKER, H. A. (1947). *J. Bact.* **54**, 381.
(16) OLSEN, A. M. & SCOTT, W. J. (1950). *Aust. J. sci. Res.* B, **3**, 219.
(17) BREWER, J. H. (1940). *J. Amer. Med. Ass.* **115**, 598.
(18) CLARK, F. M. (1943). *Food Res.* **8**, 327.
(19) WYNNE, E. S. & FOSTER, J. W. (1948). *J. Bact.* **55**, 61.
(20) WEINBERG, M., NATIVELLE, R. & PRÉVOT, A. (1937). *Les Microbes Anaérobies*. Paris: Masson et Cie.
(21) MCCLUNG, L. S. & MCCOY, E. (1934). *J. Bact.* **28**, 267.

(22) Crossley, E. L. (1941). *J. Soc. chem. Ind., Lond.*, **60**, 131.
(23) Stevens, M. K. Private communication.
(24) Andersen, A. A. (1951). *J. Bact.* **62**, 425.
(25) Miles, A. A. & Misra, S. S. (1938). *J. Hyg., Camb.*, **38**, 732.
(26) Ingram, M. & Robinson, R. H. M. (1951). *Proc. Soc. appl. Bact.* **14**, 62.
(27) Bordet, J. & Renaux, E. (1930). *Ann. Inst. Pasteur*, **45**, 1.
(28) Hardwick, W. A., Guirard, B. & Foster, J. W. (1951). *J. Bact.* **61**, 145.
(29) Hirsch, A. & Grinsted, E. (1951). *J. Dairy Res.* **18**, 198.

(*MS. received for publication* 15 *July* 1953)

637.333.12

# 544. FACTORS INFLUENCING THE LACTIC ACID-PRODUCING PROPERTIES OF STREPTOCOCCI USED IN THE MANUFACTURE OF CHEDDAR CHEESE

## I. OBSERVATIONS RELATING INHIBITORY AND STIMULATORY PHENOMENA

By G. R. JAGO

*School of Dairy Technology and Research Laboratories, Department of Agriculture, Victoria, Australia*

(With 1 Figure)

### INTRODUCTION

The lactic acid-producing properties of many single-strain starter streptococci became markedly depressed when introduced into Victorian cheese milk, pasteurized by the H.T.S.T. method, during the late winter of 1952. However, a few strains of starter streptococci showed resistance to this inhibition. The affected cultures were so slow in the cheese vats as to make their employment in the cheese factories undesirable for a period of the year.

On investigation it was found that inhibition of the affected cultures became more pronounced when these organisms were cultured in raw unheated milk. However, when the same raw milk was autoclaved or raised to boiling-point for 30 min., the inhibitory effect was completely removed. The unaffected cultures were able to produce lactic acid to the same degree whether cultured in heated or unheated milk.

It was established by inquiry that this inhibitory effect in raw and partially heated milk was widespread throughout Australia and New Zealand. Similar phenomena had been reported by Hammer & Baker (1) in America who found that milk heated at 68·2° C. for 30 min. developed acid and coagulated more slowly when inoculated with butter cultures than milk heated at higher temperatures. Farmer & Hammer (2) observed that butter cultures showed considerable variation in their capacity to withstand the so-called germicidal property of milk. They noted that six of the eight cultures studied by them were delayed in acid development when grown in raw milk or in milk pasteurized at either 62·5 or 68° C. for 30 min. Pasteurization at 71° C. for 30 min. was as satisfactory in eliminating the so-called germicidal effect as heating at 82° C. for the same period. Two of the cultures developed acid and coagulated the milk at about the same rate whether it was raw or pasteurized milk.

A number of other investigators have also reported related observations. Rice (3) in Australia, for example, noted that with lactic acid-producing streptococci, acid is formed almost as rapidly in milk heated momentarily to 76·6° C. as at higher temperatures and for longer exposures, but there is a slower rate of fermentation in milk heated to 62·8° C. for 30 min. and in unheated fresh milk. Singh & Laxminarayana (4) in India found that pasteurization and boiling of the milk provided a better medium for the growth and activity of starter cultures than did raw milk. Knudsen & Sörensen (5) in Denmark

suggested that such heating to high temperatures so modified milk that it provided a better medium for the growth of lactic acid bacteria. More recently, Czulak & Meanwell(6) in England observed a seasonal depression of activity with certain single-strain starter cultures, when these organisms were cultured in H.T.S.T. pasteurized milk. This depression was removed by boiling the milk momentarily. They also found that when a small percentage of the raw milk was added to autoclaved milk the activity of these cultures was reduced. They selected cultures for cheese manufacture on the basis of their resistance to this seasonal inhibition.

It is surprising, therefore, in view of the widespread appreciation of the increased lactic acid production in heated as opposed to raw milk, that no satisfactory explanations have been offered as to the underlying cause. The urgent requirement of establishing the nature of the inhibition of the 'susceptible' cheese starters led to a more detailed examination of the phenomenon. As a result of this investigation it has been concluded that normal milk contains not only substances which are inhibitory to 'susceptible' organisms but also substances which are stimulatory to all lactic streptococci. The observations which lead to the above conclusions are described in the present paper. In addition, an approach has been made to the problem of localizing and concentrating these two substances as a necessary preliminary to their identification.

## EXPERIMENTAL AND RESULTS

Milk used for experimental purposes was collected from a mixed herd of pure-bred Friesian and Red Poll cows numbering about ninety in the milking herd. The individual cows were in varying stages of lactation.

Where used, pasteurization was carried out by the H.T.S.T. method in which the milk was heated to a temperature of 72·7° C. for 15 sec. When autoclaved, milk was raised to a pressure of 15 lb./sq.in. (121° C.) and held at that pressure for 5 min.

Lactic acid production was determined by titrating the samples with 0·1 N-NaOH after incubation at 30° C. for 6 hr. The total titration was expressed as lactic acid, and the lactic acid as a percentage of the sample taken. Varying inocula were used, ranging from 1 to 10%.

The cultures studied were strains of *Streptococcus cremoris* and *Str. lactis*, which are used extensively in cheese factories throughout Australia and New Zealand for the manufacture of cheddar cheese. There is no phage relationship between these cultures when cross-tested against stock bacteriophages specific to each strain, except in the case of ML4 and C3. These two cultures are phage related.

Cultures are given local names for purposes of consistency and simplicity of handling in factories. A brief history of the cultures used in this work is given in Table 1.

### *Inhibition of lactic acid production*

The depression of 'susceptible' strains was first observed at the Research Station, Werribee, Victoria, when a group of single-strain cultures from New Zealand were given activity trials in the cheese vats prior to distribution to Victorian cheese factories. No obvious reason could be advanced for their slowness in the vats when compared with cultures brought from England and used by the cheese industry during the previous season with marked success.

Table 1

| Strains | Local designation | Immediate origin |
|---|---|---|
| *Streptococcus cremoris* | ML1 | New Zealand |
| | R1 | New Zealand |
| | K | New Zealand |
| | HP | New Zealand |
| | R6 | New Zealand |
| | ML4 | New Zealand |
| | E8 | New Zealand |
| | KH | New Zealand |
| | C1 | England |
| | C3 | England |
| *Streptococcus lactis* | C2 | England |
| | C10 | England |

During the vat trials it was noted that some cultures were more depressed in their lactic acid-producing properties than others, suggesting that cultures had varying degrees of susceptibility to an inhibitory substance. This is shown in Table 2, which gives the percentage of lactic acid produced in raw, H.T.S.T. pasteurized and autoclaved milk taken from the same bulk of mixed milk.

Table 2

| | % lactic acid produced in milk when | | |
|---|---|---|---|
| | Raw | H.T.S.T. pasteurized | Autoclaved |
| | Susceptible cultures | | |
| ML1 | 0·31 | 0·31 | 0·71 |
| R1 | 0·36 | 0·42 | 0·71 |
| K | 0·32 | 0·33 | 0·72 |
| HP | 0·33 | 0·33 | 0·73 |
| R6 | 0·49 | 0·48 | 0·75 |
| ML4 | 0·41 | 0·47 | 0·73 |
| E8 | 0·51 | 0·51 | 0·71 |
| KH | 0·36 | 0·41 | 0·73 |
| | Resistant cultures | | |
| C1 | 0·75 | 0·77 | 0·73 |
| C2 | 0·71 | 0·70 | 0·70 |
| C3 | 0·73 | 0·76 | 0·75 |
| C10 | 0·72 | 0·73 | 0·73 |

Furthermore, inhibition was most pronounced during the cheddaring process when rapid shrinkage and matting of the curd takes place, and when the multiplication of the organisms is most rapid. Table 3 shows a comparison between the activities of a 'resistant'

Table 3

| Time interval from cutting (hr.) | % lactic acid produced | |
|---|---|---|
| | 'Resistant' culture (C2) | 'Susceptible' culture (ML1) |
| 0 (vat cut) | 0·090 | 0·090 |
| ½ | 0·090 | 0·090 |
| 1 | 0·100 | 0·100 |
| 1½ | 0·105 | 0·105 |
| 2 | 0·120 | 0·110 |
| 2½ | 0·140 | 0·125 |
| 3 (vat dried) | 0·220 | 0·140 |
| 3½ | 0·340 | 0·170 |
| 4 | 0·480 | 0·210 |
| 4½ (vat milled) | 0·640 | 0·260 |

culture (C2) and a 'susceptible' culture (ML1) when cultured in the cheese vat under identically the same conditions. Whey acidities were determined at half-hourly intervals after 'cutting'. The bulk starter acidity of both cultures prior to addition to the cheese milk was 0·650%. By contrast, as shown in Table 2, the lactic acid production for cultures C2 and ML1 in autoclaved milk was 0·70 and 0·71% respectively.

Many reports came to hand of slowness when these 'susceptible' cultures were supplied to cheese factories in various districts. One factory situated on the north coast of New South Wales, and approximately 1000 miles from the Research Station, supplied weekly

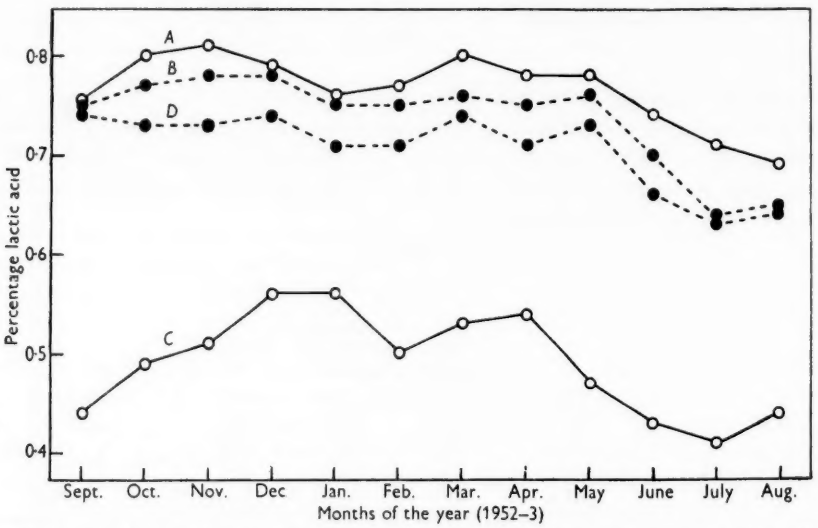



**Fig. 1.** Variation in lactic acid production by 'resistant' and 'susceptible' starter streptococci when cultured in raw and boiled milk. The latter held at 100° C. for 20 sec. The percentage lactic acid was determined weekly for each organism after incubation for 6 hr. at 30° C. using 9% inoculum. Mean values for the eight 'susceptible' and two 'resistant' cultures were obtained for each four-weekly period, and these feature as the experimental points on the graph.

Curves *A* and *B* are results for the 'susceptible' and 'resistant' strains in boiled milk; curves *C* and *D* for the same organisms in raw milk.

data after testing their cultures in raw, laboratory-pasteurized (72·7° C., 15 sec.) and boiled milks (100° C., 20 sec.). This factory had been using 'susceptible' cultures exclusively and had not become aware of their slowness in the cheese vats until supplied with 'resistant' cultures for comparison. They noted a marked contrast in activity between 'susceptible' and 'resistant' cultures when these were used in the cheese vats.

Mean monthly figures obtained by this factory for a 12-month period are shown in Fig. 1 to illustrate the seasonal variation in lactic acid production as observed in raw and boiled milks. Results are given for both 'resistant' and 'susceptible' organisms. It is seen that, while there is some variation in lactic acid production, the inhibition of the susceptible organisms is consistently maintained throughout the 12-month period.

In experiments at Werribee it was found that the addition of 10% of raw milk containing the inhibitory factor was sufficient to depress the lactic acid production of the 'susceptible' organisms when grown in autoclaved milk. The inhibitory effect was abolished by heating the raw milk to 100° C. for 30 min., or to 121° C. (15 lb. pressure/sq.in.)

for 5 min. However, the minimum heating conditions for destruction of the inhibitory factor have not as yet been determined. These will be reported at a later date.

As the number of phage-unrelated 'resistant' cultures for distribution are limited, it became imperative to consider some practical means of overcoming the slowness exhibited by 'susceptible' cultures in the cheese vats. At the present time the only method of doing this is to select cultures with the least susceptibility to inhibition and to increase the amount of bulk starter (inoculum) added to the cheese vat. It is important to note that, where the same conditions of manufacture are required to be maintained, the amount of bulk starter to be added in the case of each culture will vary according to the degree of inhibition of each individual culture in unheated milk. Table 4 shows that by increasing the inoculum in raw unheated milk, lactic acid production can be increased over the same time interval. Lactic acid production in the same milk when autoclaved is given for comparison.

Table 4

| | % lactic acid produced by 'susceptible' culture ML1 in | |
|---|---|---|
| % inoculum | Raw (unheated) milk | Autoclaved milk |
| 0·001 | 0·18 | 0·18 |
| 0·01 | 0·20 | 0·21 |
| 0·1 | 0·20 | 0·27 |
| 1·0 | 0·39 | 0·54 |
| 5·0 | 0·51 | 0·71 |
| 10·0 | 0·60 | 0·82 |

At first it was thought that milk carrying this inhibitory substance may be produced only by isolated cows. Random selection of twenty cows from the farm herd gave results which suggested its presence in the milk of all cows. It was present in the milk drawn from individual quarters of the udder and in milk drawn aseptically, thereby dissociating its presence from metabolites produced by organisms contaminating the milk after ejection. It was present in milk drawn from cows of all breeds. During a short trial it could not be shown that the type of feed fed to a cow could explain the presence of this factor in the milk.

When cream was removed from raw whole milk, 'susceptible' cultures showed a marked increase in lactic acid production. Heating the skim milk gave a still further increase in lactic acid production. These two effects are clearly shown in Table 5.

Table 5

| 'Susceptible' culture ML1 cultured in | % lactic acid |
|---|---|
| Whole milk (raw, unheated) | 0·31 |
| Skim milk (raw, unheated) | 0·53 |
| Skim milk (autoclaved) | 0·68 |
| Whole milk (autoclaved) | 0·69 |

The addition of cream and raw whole milk to autoclaved skim milk in increasing amounts, as shown in Table 6, resulted in a depression of activity which was more pronounced in the case of cream.

From Tables 5 and 6 it is apparent that the inhibitory substance is largely associated with the fat globules, possibly adsorbed on to their surfaces. In support of this, after churning the cream, buttermilk was found to be inhibitory, while the fat granules were inert in their effect upon 'susceptible' organisms.

Table 6

| Amount of raw whole milk added to 9 ml. autoclaved skim milk (ml.) | % lactic acid | Amount of cream added to 9 ml. autoclaved skim milk (ml.) | % lactic acid |
|---|---|---|---|
| 0 | 0·54 | 0 | 0·54 |
| 0·1 | 0·47 | 0·1 | 0·45 |
| 0·2 | 0·43 | 0·2 | 0·38 |
| 0·3 | 0·41 | 0·3 | 0·31 |
| 0·4 | 0·38 | 0·4 | 0·29 |
| 0·5 | 0·36 | 0·5 | 0·26 |

*Stimulation of lactic acid production*

While considering the adsorption effect of the inhibitory substance on the fat globule, it was thought desirable to see whether the same substances could be located on the small particles removed from the milk in the separator slime. The slime was emulsified in Ringer's solution and divided into two portions, one portion being autoclaved to destroy the inhibitory substance if it should be present. However, instead of an inhibitory effect being obtained, a marked increase in lactic acid production occurred in both samples. It made no difference whether the slime was heated or unheated in producing this effect. Table 7 shows the effect of heated slime when added to raw and autoclaved whole milk.

Table 7

| Amount of heated slime added (ml.) | % lactic acid produced by 'susceptible' culture (ML1) | |
|---|---|---|
| | Raw whole milk | Autoclaved whole milk |
| 0 | 0·26 | 0·45 |
| 1 | 0·40 | 0·70 |
| 2 | 0·50 | 0·87 |
| 3 | 0·66 | 0·96 |

Equal weights of separator slime and Ringer's solution mixed and boiled for 5 minutes. Volumes indicated added to 9 ml. of milk.

It is quite clear from Table 7 that stimulation of the organism's capacity to produce lactic acid is much less in the case of raw milk containing separator slime. This seemed to suggest that the inhibitory substance was interfering with the utilization by the organisms of the growth-promoting substances normally present in milk. Further proof of this was obtained when freshly separated cream was added in increasing amounts to autoclaved skim milk containing an infusion of separator slime as shown in Table 8.

Table 8

| Amount of raw cream added to autoclaved skim milk containing separator slime (ml.) | % lactic acid produced by 'susceptible' culture (ML1) |
|---|---|
| 0 | 0·88 |
| 0·1 | 0·83 |
| 0·2 | 0·77 |
| 0·3 | 0·74 |
| 0·4 | 0·65 |
| 0·5 | 0·51 |

20 ml. of slime suspension (see Table 7) added to 70 ml. milk and dispensed in 9 ml. quantities before addition of cream as shown.

Moreover, by the addition of more cream or more separator slime, one could vary the amount of lactic acid produced. This suggested that inhibition of the 'susceptible' cultures may be of a competitive nature.

Separator slime is known to be a material of some complexity, and the manner in which it stimulates lactic acid production by lactic streptococci is, at present, under investigation.

In order to determine whether other material would overcome the inhibitory effect, an infusion of yeast extract was added to raw milk. It is well known that yeast extract will greatly increase the level of lactic acid production in milk by starter streptococci. The same effect was produced on the 'susceptible' organisms as in the case of the addition of separator slime to the milk. Similarly, as before, the level of lactic acid production in autoclaved milk to which yeast extract had been added was depressed on the addition of cream.

It is known that liver extracts have, for some organisms, similar growth-promoting properties to that of yeast extract. A liver extract, prepared by grinding the liver of a domestic fowl in Ringer's solution, was added to raw whole milk. There resulted an increase in lactic acid production by the 'susceptible' organisms as shown in Table 9. Furthermore, heating of the extract to 100° C. or autoclaving at 15 lb. pressure increased its growth-promoting properties. This increase of activity, shown in Table 9, is due possibly to the effect of heat on the tissue cells, thereby destroying the cells and liberating additional growth-promoting substances.

Table 9

| Amount of liver extract added (ml.) | % lactic acid produced by 'susceptible' culture (ML 1) | | |
|---|---|---|---|
| | Unheated liver extract plus raw whole milk | Heated liver extract plus raw whole milk | Unheated liver extract plus autoclaved whole milk |
| 0 | 0·26 | 0·26 | 0·56 |
| 1 | 0·29 | 0·30 | 0·75 |
| 2 | 0·33 | 0·38 | 0·86 |
| 3 | 0·36 | 0·46 | 0·94 |
| 4 | 0·40 | 0·55 | 1·00 |

Liver extract prepared by grinding 1 g. of fresh liver in 2 ml. of Ringer's solution. Amounts indicated were added to 9 ml. of milk.

Again, as in the case of separator slime and yeast extract, when cream was added in increasing amounts to autoclaved skim milk containing an infusion of liver extract, a decrease of lactic acid production occurred as shown in Table 10.

Table 10

| Amount of raw cream added to autoclaved skim milk containing liver extract (ml.) | % lactic acid produced by 'susceptible' culture (ML 1) |
|---|---|
| 0 | 0·71 |
| 0·1 | 0·67 |
| 0·2 | 0·54 |
| 0·3 | 0·48 |
| 0·4 | 0·43 |
| 0·5 | 0·40 |

10 ml. of liver extract (see Table 9) was added to 80 ml. of milk and dispensed in 9 ml. quantities before addition of cream as shown.

Incubation of a 'susceptible' culture in raw whole milk for a prolonged period brought about coagulation of the curd and development of acidity to the same level as reached by the same organism in autoclaved milk with a much-reduced period of incubation. This suggests that the inhibitory substance is bacteriostatic in its effect, and not bactericidal. Additional evidence in support of this bacteriostatic effect is provided by following the growth and multiplication of the starter organisms in the maturing cheese. A 'susceptible' culture will take much longer to reach its maximum population in the cheese than will a 'resistant' one.

Many workers[3,4,7,8,9] have reported reduced activity of starter organisms when cultured in mastitis milk. Samples of milk were drawn aseptically from the four quarters of a cow, one of which was infected with mastitis. Both 'resistant' and 'susceptible' organisms were cultured in the raw samples and in the same samples autoclaved. The results, as shown in Table 11, suggest a reduction of the growth-factor level in milk from the affected quarter, as can be seen by the reduced activity of the 'resistant' culture C2. However, work on this phenomenon is proceeding.

Table 11

| Section of udder from which samples drawn | % lactic acid produced by 'Resistant' culture (C2) Raw whole milk | 'Resistant' culture (C2) Autoclaved whole milk | 'Susceptible' culture (ML1) Raw whole milk | 'Susceptible' culture (ML1) Autoclaved whole milk |
|---|---|---|---|---|
| Left fore quarter | 0·54 | 0·53 | 0·26 | 0·50 |
| Left rear quarter | 0·51 | 0·51 | 0·25 | 0·52 |
| Right fore quarter | 0·54 | 0·55 | 0·30 | 0·51 |
| Right rear quarter (mastitis) | 0·42 | 0·42 | 0·24 | 0·17 |

During the course of these investigations it was found that milk on standing in the refrigerator gradually lost its capacity to promote high lactic acid production when the same culture was inoculated from day to day. As this phenomenon was more pronounced in the case of 'resistant' cultures it was assumed that some factor, possibly enzymic, was destroying the essential growth factors for this organism.

## DISCUSSION

The results shown in Tables 2–6 indicate that milk normally contains a heat-labile inhibitory substance, closely associated with the fat globule, which restricts lactic acid production by certain strains of lactic streptococci. In addition, milk contains heat-stable substances which, if present in sufficient quantity, can overcome the effect of the inhibitory substance against these cultures and which is stimulating to all strains of starter streptococci. This is clear from consideration of Tables 7–10. The resultant lactic acid production in milk by 'susceptible' starter streptococci will be dependent upon the ratio of the amounts of these two substances in the milk. However, there are cultures which are resistant to, or nutritionally independent of, this inhibitory substance and whose activity in milk will largely be controlled by the amount of stimulatory substance present.

These findings appear to disagree with the theory advanced by Knudsen & Sörensen[5] who stated that the products of decomposition, formed at high heating temperatures, may be responsible for the increased activity of starter organisms in heated milk. They

considered that the bacteria were able to utilize these substances better than those constituents occurring naturally in the milk. However, they also stated that exposure of the milk to high temperatures may result in the destruction of bactericidal substances.

Support is given to the theory advanced by Czulak & Meanwell(6) who stated, after using a well-defined synthetic medium, that inhibition of 'susceptible' organisms may be related to their inability to synthesize some growth-stimulating factor which the 'resistant' cultures were able to synthesize for themselves. They advanced the hypothesis that inhibited cultures have a reduced activity in winter because an unknown growth factor which they require is at that season made unavailable by a heat-labile inhibitory substance. Czulak & Meanwell suggested that in summer, when the cows are fed mainly on grass and green fodder, the concentration of the growth factor is sufficiently high to overcome the effect of the inhibitor. On the other hand, 'resistant' cultures synthesize their own growth factor in sufficient quantity so that they are independent of the inhibitor effect at all times. These workers also suggested that the ability to synthesize the essential growth factor enabled resistant organisms to grow in a synthetic medium in which the 'susceptible' cultures failed to develop. However, no experimental work has been reported by these authors to substantiate these very interesting hypotheses.

Certain terms used throughout this paper have been employed somewhat loosely through lack of a clearer understanding of certain phenomena which it is hoped further research, at present being conducted, will provide. As the title to the paper suggests, it has largely been a series of observations concerning lactic acid production in milk under varying conditions and at different periods of the year. Growth factors have been frequently referred to in relation to lactic acid production. So far it has been assumed that growth and reproduction of the organism is linked with lactic acid production, and if a high level of lactic acid is obtained in the milk it is because the factors required for growth and reproduction of the organism are present in the milk in greater quantity. Slight evidence supporting this assumption was only obtained by studies under the microscope which clearly showed a marked increase in chain length (in the case of *cremoris* strains) and cell size, when growth-stimulating factors present in separator slime, liver and yeast extract were added to the milk.

As a result of observations made both in New South Wales and Victoria, there appears to be a seasonal variation in the amount of stimulatory material present in normal milk supplies. This is indicated in the accompanying figure by the fluctuation in lactic acid produced by both 'susceptible' and 'resistant' strains when grown in boiled milk. In general, a decline in the level of this factor appears to occur during the summer months (January and February). As shown in the figure, however, late summer rains in New South Wales restored the level to a new peak in March. From these considerations it would appear necessary to adjust the amount of bulk starter added to the cheese vat according to:

(*a*) the degree to which an organism is inhibited in raw or pasteurized milk,

(*b*) the period of the year (or state of pasture growth).

It is noteworthy that some organisms are more susceptible to this inhibitory factor than others. Undoubtedly this difference is related to the nutritive requirements of the different starter strains. It appears to be a definite characteristic of the organism which remains constant. Susceptibility cannot be entirely related to *cremoris* strains, as there are some strains which are resistant to this factor. So far the work has not been extended

to cover a survey of *lactis* strains, but it seems reasonable to suppose that both 'resistant' and 'susceptible' strains would be found. Indeed, since the initial stages of this work were completed, a 'susceptible' strain of culture C10 (*Streptococcus lactis*) has been identified. This culture was formerly thought to be entirely 'resistant'.

It is of interest to note the relationship between ML4 and C3. These two organisms are attacked by the one phage, but their susceptibility to the inhibitory substance is quite different, ML4 being susceptible while C3 is resistant. Recently another culture was isolated which is phage-related to culture R6 but shows greater resistance to inhibition than this organism.

In order to interpret some of the observations reported in this paper, it will clearly be necessary to have a more detailed understanding of the nature of the inhibitory and stimulatory materials present in milk. The behaviour of the three growth-promoting substances, separator slime, fowl liver and yeast extract, in milk; the manner in which they stimulate the growth and lactic acid-producing properties of all starter cultures; and the manner in which their effects are suppressed on the addition of cream (in the case of 'susceptible' cultures) would suggest that they contain a common substance which is essential for the growth of all starter organisms. It appears that this material can be made unavailable to certain organisms by the action of a substance normally present in milk and associated with the fat globules. It is of some interest that Wilson & Rosenblum (10) and, more recently, Auclair & Hirsch (11) have reached somewhat similar conclusions in relation to the action of 'lactenin' on streptococci of a large number of different antigenic groups, but excluding group N (lactic streptococci). It is with this latter group that the present investigation has been directly concerned. It is noteworthy that neither Wilson & Rosenblum (10) nor Auclair & Hirsch (11) reported association of the inhibitory factor with milk-fat globules.

The competition between the stimulatory material and the heat-labile inhibitory substance complicates the interpretation of some of the present findings. Considering the heat lability of the inhibitory substance and the manner of its association with the fat globules (where an enormous surface area is exposed in milk) it may well prove to be enzymic in nature. The association of a number of enzymes with sub-microscopic lipoprotein particles ('microsomes'), which are strongly adsorbed to the milk fat globules, has recently been established by Morton (12). In collaboration with Dr Morton, investigations into the relationship of the inhibitory substance with the fat globule, as well as identification of both the inhibitory and stimulatory materials, are now proceeding. When the nature of these materials is established, it may well be possible to overcome the apparent nutritional deficiency of the 'susceptible' strains and to stimulate lactic acid production by all starter organisms in cheese milk. It is quite evident that milk, as normally secreted by the cow, is a relatively poor medium from the point of view of supporting lactic acid production by starter streptococci.

## SUMMARY

1. Many single-strain lactic streptococci used as cheese starters exhibited a difference in susceptibility to an inhibitory substance present in raw and H.T.S.T. pasteurized milk, some being markedly affected whilst others were completely resistant to this inhibitory substance.

2. The inhibitory effect of raw or partially heated milk on 'susceptible' strains of starter streptococci appears to be a widespread phenomenon. It can be said that the inhibitory substance is normally present in all milk supplies throughout the year. It occurs in milk freshly drawn from the udder aseptically.

3. Heating the milk to boiling-point for variable periods or autoclaving the milk destroys the inhibitory effect of the milk on 'susceptible' cultures.

4. The inhibitory substance is closely associated with the fat globules in milk and can exert a marked effect owing to the large surface area exposed.

5. Growth-stimulating substances prepared from extracts of fowl liver, separator slime, and yeast were able to overcome the inhibitory effect when added to raw milk. However, the effect of these substances were markedly depressed on the addition of small amounts of freshly separated cream to the cultures.

6. It is suggested that the above growth-stimulating substances contain a common growth factor which enters into competition with an inhibitory substance closely associated with the fat globule.

7. The inhibitory substance appears to be bacteriostatic in its effect and not bactericidal.

8. Owing to the heat lability of the inhibitory substance and its presence in close association with the fat globule, it is suggested that it may be enzymic in nature.

I wish to thank Mr J. Czulak for his interest in this work, in supplying cultures, and providing information from his recent tour of New Zealand. I also wish to offer my sincere thanks to Dr R. K. Morton for helpful advice and constructive criticism of this work, and to Mr M. Wojciechowicz for his assistance in the laboratory.

In conclusion, I would like to thank Mr M. Swinbourne, Technologist of Norco Co-op. Ltd., for his kindness in supplying estimates of activities of various starters over a 12-month period, and for permission to report these results. This information was obtained often at much inconvenience to himself.

## REFERENCES

(1) HAMMER, B. W. & BAKER, M. P. (1928). *Res. Bull. Ia agric. Exp. Sta.* no. 106, p. 148.
(2) FARMER, R. S. & HAMMER, B. W. (1931). *Res. Bull. Ia agric. Exp. Sta.* no. 146, p. 21.
(3) RICE, E. B. (1942). *J. Aust. Inst. agric. Sci.* **8**, 28.
(4) SINGH, K. & LAXMINARAYANA, H. (1948). *Ind. J. Dairy Sci.* **1**, 78.
(5) KNUDSEN, S. & SÖRENSEN, A. (1929). *Zbl. Bakt.* II. Abt. **79**, 75.
(6) CZULAK, J. & MEANWELL, R. J. (1951). *Proc. Soc. appl. Bact.* **14**, 1.
(7) DAVIS, J. G. & MCCLEMONT, J. (1939). *J. Dairy Res.* **10**, 94.
(8) DAVIS, J. G. & MATTICK, A. T. R. (1936). *Agric. Progr.* **13**, 126.
(9) LEITCH, R. H. (1937). *Agric. Progr.* **14**, 40.
(10) WILSON, A. T. & ROSENBLUM, H. (1952). *J. exp. Med.* **95**, 25 and 39.
(11) AUCLAIR, J. E. & HIRSCH, A. (1953). *J. Dairy Res.* **20**, 45.
(12) MORTON, R. K. (1953). *Nature, Lond.*, **171**, 734.

(*MS. received for publication* 21 *September* 1953)

637.0:54

# REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

## SECTION C. DAIRY CHEMISTRY AND PHYSICS

PART I. CHEMISTRY AND PHYSICAL CHEMISTRY

I. Composition of milk and milk products:
(*a*) Milk
(*b*) Butter
(*c*) Cheese

II. Chemistry of milk constituents:
(*a*) Fat
(*b*) Nitrogenous compounds
(*c*) Miscellaneous
(*d*) Enzymes

III. Physical Chemistry:
(*a*) Proteins
(*b*) Other aspects

IV. Defects of milk and milk products:
(*a*) Adulteration and its detection
(*b*) Flavour defects

V. Analysis of milk and milk products:
(*a*) Milk
(*b*) Butter
(*c*) Cheese
(*d*) Dried milk, etc.

References:
(*a*) Arranged numerically
(*b*) Arranged alphabetically

PART II. PHYSICS (RHEOLOGY)

## PART I. CHEMISTRY AND PHYSICAL CHEMISTRY

This review deals largely with the progress of dairy chemistry from the middle of 1950 to the end of 1952. It includes earlier publications not covered by the previous Review (1).

### I. COMPOSITION OF MILK AND MILK PRODUCTS

#### (*a*) *Milk*

*Cow's milk*

Further investigations of the properties of colostrum (2) indicate that the rapid fall in cream volume and viscosity during the first few milkings is related to the decrease in specific gravity, non-fatty solids, and albumin-globulin concentration. Italian workers (3,4,5,6) examined the effect of heavy injections of vitamin $D_2$ (4,800,000 i.u.) on the composition of colostrum. Three injections were given and a marked retardation of the normal transitional decline in specific gravity, and protein, was observed in groups injected pre-partum and post-partum. Acidity and chlorine values were similarly affected by pre-partum treatment only. The retarding effect on fat percentage was effected by post-partum injections only. The protein/fat ratio tended to increase in the pre-partum treated group and to decrease in the group not treated until after parturition. The chlorine/lactose ratio declined less rapidly before parturition and more rapidly after parturition as a result of pre-partum injections.

Rowland, Roy, Sears & Thompson (7) also examined the composition of pre- and post-partum secretions of eleven shorthorn cows which were 'steamed up' with 4–8 lb. concentrates for 37 days and hand milked daily for 16 days before calving. While the actual weights of all constituents increased daily during the pre-partum period, globulin showed a peak at the time of rapid increase in milk yield, suggesting that globulin passes from the blood stream at this stage under hormonal stimulus. Vitamin A and carotenoid content were positively correlated to globulin content. Part of these constituents appears to be associated with a lipid appearing in the udder at about the same time as globulin. The concentrations of those constituents which are characteristically high in normal colostrum were even higher in the earliest pre-partum secretion. The concentration of non-fatty solids, protein, ash and phosphorus in post-partum secretion of pre-milked cows was significantly lower than that of normal colostrum; the lactose concentration was significantly higher. Colostrum was shown by Pope & Roy (8) to contain oestrogens in concentrations approximately equal to those of bovine blood in pregnancy. They are mainly in the aqueous phase, some being ketonic. Normal milk has a negligible oestrogenic activity.

The slight but significant downward trend in non-fatty solids and fat of milk reaching the English consumer has been the subject of many articles and reviews (Provan(9), Rowland(10), Bartlett & Kay(11), Davis(12)). In the latter, weighted averages of very large numbers of samples show that the non-fatty solids have declined from 8·91% in 1931–5 to 8·74% in 1946–50, while fat has fallen from 3·70% in 1926–30 to 3·62% in 1946–50. Further investigation into previously recorded influence of quantity of roughage on milk secretion by Balch, Balch, Bartlett, Cox & Rowland(13) has shown that reduction of the hay ration from 18 to 6 lb. and 2 lb. led to significantly lower fat percentages but had no effect on the non-fatty solid content. The effect was temporary—both the low roughage groups returning to normal in the post-experimental period. The low roughage rations gave a milk fat with a lower softening point and increased iodine value. The fat from the 2 lb. but not the 6 lb. group had a lower Reichert value. Saponification values were unaffected. Milk yields increased in the 6 lb. group but decreased in the 2 lb. group. In both these groups compensation for the loss of roughage was made by addition of a concentrate mixture low in fibre and protein. Bailey(14), examining data from a series of investigations at the National Institute for Research in Dairying during the period 1935–46, found that milk yield rose to a maximum for sixth calvers and milk quality was highest for first calvers. There was no correlation between lactation yield and solids-not-fat. The correlation between lactation butterfat and solids-not-fat was rather low, $r = +0{\cdot}40$ approx., from which the author claims that a breeding policy to improve milk quality should not be based on butterfat records alone. From the middle of the second month to the middle of the sixth month of lactation a steady recovery from the initial fall in non-fatty solids was evident, the gradient being greatest for young animals. The solids-not-fat level rose from April to May, fell from May to July and rose again from August to September. No constant trend was observed during the period December to March. During the 12-year period examined a significant fall of 0·25% solids-not-fat was observed in the milk of first calvers. For the months February and March during this 12-year period the solids-not-fat content of the milk varied directly with the amount of starch equivalent and inversely with the dry-matter content of the diet. Since the starch equivalent showed little change over the 12-year period, it is suggested that the decline in solids-not-fat may be due to greater dry-matter intake(15), i.e. a greater weight of food required to supply the necessary lb. starch equivalent. Mahadevan(16) studied the effect of environment and heredity on milk yield, persistency of lactation, and fat percentage from about 5000 180-day lactation records from twelve pedigree Ayrshire herds. Winter calvers produced about 10% more milk than summer calvers. The yield was influenced both by the number of previous lactations and age at calving. The probable performance of a cow in any lactation was predicted as accurately from the preceding lactation as from the average of a number of lactations. A satisfactory numerical expression for the shape of the lactation curve was obtained from the formula: persistency $= (A - B)/B$, where $A =$ 180-day lactation yield and $B =$ the initial yield during the first 10 weeks. First calvers had a significantly higher persistency than later calvers. Fat percentage was only very slightly affected by month of calving, age, or calving interval. Edwards(17) finds that the a.m. milk and fat yields are adversely affected by udder pressure during the first half of the lactation. Evidence is presented to show that the 'pressure hold-over' theory does not explain the difference between a.m. and p.m. fat percentages, nor does the greater volume of milk account for the lowered fat percentage of morning milk. There was a fundamental inverse relationship between milk volume and fat content. These observations arose from a study of 1766 Jersey Register of Merit lactation records and 857 records of cows in a pedigree Jersey herd.

In a study of variations in composition of milk from twelve milk-recorded cows during the period 28 February to 23 May 1950, Featherstone, Rickaby & Cavell(18), in this country, found that although feeding and management were satisfactory, the non-fatty solids remained below 8·5% until 24 April, when an increase in milk protein corrected the deficiency. This recovery coincided with spring grass feeding. Low concentrations of non-fatty solids during a bad winter are reported from New Zealand(19). A predominance of Friesians, and insufficient concentrate feeding are cited as contributory causes.

Changes in milk composition resulting from the change from stall feeding to grazing were examined by Kublitz (20) in Germany. While lactose concentration remained fairly constant, both fat and protein declined in the spring and rose again in the autumn, the changes occurring 2–3 weeks after the transfers. Similar changes in fat percentage occurred in herds kept wholly indoors and were attributed to changes in the protein/starch ratio of their food. Seasonal changes are also reported from Brisbane (21). A significant decline in composition was noted in late winter and early spring when the pastures were poor. The low fat percentages in the morning milk during late winter were attributed to the long night interval rather than seasonal influences. Kielbasińska (22) found only a slight seasonal variation in non-fatty solids of several thousand samples of milk in the Lodz area, the highest values being 8·95 to 9·08% for November–February and the lowest 8·70 to 8·77% for June–September.

The protein-fat relationship was examined by Antoine (23) in ninety-one bulk samples from nine cows during the months January to June; a significant positive correlation $r = 0{\cdot}637$ was found. The subject was also studied by Lonka (24), and by Stüber & Göbelt (25). The latter state that while there is a relationship between fat and protein, it is not sufficiently constant to permit an assessment of the protein content from the fat percentage.

The effect of interruption of milking on the protein fractions of milk was to increase the percentages of globulin, albumin and non-protein nitrogen (26). In this work the twice daily milking of four Friesians and one Jersey cow was discontinued for 10 days. The authors suggest that post-interruption milk may provide a product with increased antibody content, suitable for the feeding of newborn calves when colostrum is not available.

Changes in fat percentage during milking were investigated by Johansson, Korkman & Nelson (27). The average difference (last to first drawn) was about 8%. Variations in the observed increase during milking showed a positive correlation with milk yield. The intra-mammary post-ejection pressure was highly correlated with both yield and fat percentage increase, but when yield was held constant, the partial correlation between pressure and rise in fat percentage was practically zero. High milk-flow rates and high average total fat percentages were also associated with a high rise in fat percentage. Greater increases were observed in the morning than in the evening milk. Vigorous massage of the udder before milking tends to distribute the fat more evenly between the first- and last-drawn portions. Massage for 5 min. had a very pronounced effect. The authors conclude that the fat globules are held up either in the alveoli or in the small ducts, and that the delay is influenced by globule size. Johansson (28) found that residual milk obtained from cows previously milked, hand-stripped and intravenously injected 5 min. later with 25–30 i.u. oxytocin, amounted to 1·1 kg. from cows averaging 7·4 kg. per ordinary milking. The fat content of the residual milk was 12·5%. Smaller quantities of residual milk were given by first-lactation heifers, from which it is concluded that young animals release their milk much more readily than older cows. Work by Korkman (29) in Sweden, about the effects of advancing lactation on fat percentage, confirms the views generally held in other countries. Similarly, the progress of lactation of 150 Siberian × Friesian crossbred cows was found to affect the concentrations of fat, protein and lactose as already observed in this and other countries (30). During a 14-month observation of thirty-three cows (31), oestrus had no constant effect, although it frequently caused an increase in fat percentage. Improvement in weather conditions usually favoured fat secretion and vice versa.

Paper chromatography has revealed the presence of glucose, galactose and glutamic acid in milk (32). A nitrogenous sugar compound was also present which yielded on hydrolysis galactose, glucose and an amino-sugar. Human milk and rat's milk also contained these compounds. The anti-oxidant properties of tocopherol in milk were observed by Krukovsky & Loosli (33). The normal winter decrease in tocopherol concentration was arrested by feeding 2 g. mixed tocopherols daily. Sharp increases in concentration occur when pasture feeding commences. Yield and fat percentage are not affected by tocopherol intake.

Experimental Q fever infection of the mammary gland was found by Ormsbee (34) to increase fat and non-fatty solid percentages. Total and non-casein nitrogen increased while casein

nitrogen decreased; chloride percentage was high, and lactose percentage low; and there was a significant fall in yield.

Schmid & Zollikofer(35) observed that the strain of a 30 km. journey of six cows to higher altitudes of the Alps caused an immediate decline in milk yield, fat and lactose percentages, while the chloride concentration increased.

Data for bulk milk received in Norwegian dairies for the three periods 1927–30, 1937–40 and 1944–5 show but little variation(36), the levels of protein being 3·11, 3·19 and 3·11%, those of lactose 4·64, 4·87 and 4·83%, and those of fat 3·72, 3·80 and 3·69% respectively. Ash remained steady at 0·72%.

The milk of six Sindhi cows and five buffaloes was examined for cholesterol by Gulvady, Kannan & Basu(37). Variations from 10 to 50 p.p.m. were noted in the former and from 10 to 80 p.p.m. in the latter. In both species there was a sharp rise at the end of lactation. Evidence of proteolysis during the souring of the milk of cows and buffaloes is presented by Khambatta & Dastur(38). Variations in non-protein nitrogenous constituents of the milk of cows, buffaloes, goats and sheep were examined by Venkatappaiah & Basu(39). All the non-protein nitrogen constituents except creatinine were 2–5 times higher in the first colostrum of cows and buffaloes. The averages were respectively 258·2, 276·0, 323·9 and 432·9 p.p.m., of which urea was 44·8, 41·2, 64·9 and 32·9 p.p.m., uric acid 2·1, 0·9, 0·6 and 0·4 p.p.m. and amino-acids 15·7, 18·6, 16·6 and 22·2 p.p.m. Bakalor(40) found that the mean composition of 1608 samples of milk from forty-six producers taken on arrival at a Pretoria milk plant was: total solids 12·12%, fat 3·51%, solids-not-fat 8·61%, ash 0·737%, protein 3·19% and lactose 4·69%. Fat and ash showed greatest variation. During the 12-month period 2·8% of the total number of samples fell below 3·0% fat, and 35·4% fell below 8·5% non-fatty solids. The milk from 25% of the producers contained less than 8·5% yearly average non-fatty solids. The author concludes that mastitis and poor feeding during the dry winter months are largely responsible for low non-fatty solid content. The Capetown city milk supply was shown by Bakalor(41) to have declined steadily in both fat percentage and solids-not-fat over the years 1934–46. In this period the fat varied between 3·51% in December to 3·79% in May, while solids-not-fat rose to 8·74% in July to September after reaching a minimum of 8·62% in January. The same author examined the milk supply of nine South African cities(42) during the period October 1949 to September 1950, and again noticed a frequent failure to reach the 8·5% limit. In some cities all samples failed in this respect for one or more months in the year. Deficiencies in fat were much less frequent. Milk of 138 cows in the Parma region of Italy showed a decline in fat percentages from 3·86 to 3·52% during the period 1939–43(43). The bulked milk of 30,000 cows in the province of Milan during the period 1930–9 was found by Fabris(44) to contain 3·49% fat and 8·87% solids-not-fat. Fat content was lowest in April (3·34%) and highest in October (3·62%), while non-fatty solids were lowest in August (8·71%) and highest in April (9·05%). The decline in fat content was also noted by Carbone(45). At the beginning of the century milk in the Milan area contained 3·7% fat, and in 1939 it had fallen to 3·49%.

The effect of grazing at night and resting by day on milk composition was investigated by Shvabe(46) in an experiment extending over a month, using six Yaroslav cows. Compared with the normal day grazing–night resting, the average figures were 12·86 kg. daily yield, 3·86% fat for the night grazing, and 11·78 kg., 3·97% respectively for the day grazing. Jack, Roessler, Abbott & Irwin(47) examined over 20,000 samples of milk produced in California, and found that the solids-not-fat content could be estimated from the fat percentage by use of the formula solids-not-fat = 7·07 + 0·444 fat (%); the standard error was 0·36.

Uesaka *et al.*(48,49) gave the following composition of the milk of brown Japanese cows: 5·49% fat, 4·36% protein, 3·55% lactose and 0·68% ash. The fat had a saponification value of 230·1, a Reichert value of 30·3, an acid value of 1·2 and an iodine value of 28·1. Melting-point 33° C. Mineral concentrations (g./l.) were: $P_2O_5$, 2·34; CaO, 2·02; $K_2O$, 1·27; Cl, 0·51; $Na_2O$, 0·48.

Weekly analyses of the milk of Ayrshire and Friesian cows over sixty complete lactations showed that the percentage of Ca and P increased with that of the fat. For a fat content of

2·68%, 0·106% Ca and 0·082% P were found, whereas 6% fat milk showed 0·180% Ca and 0·098% P(50). Munch-Petersen found the copper content of fifty-one samples of fresh milk to be 0·0553 p.p.m. ± 0·0046, of bottled raw milk 0·0820 p.p.m. ± 0·0084, and of heat-treated bottled whole milk 0·0995 p.p.m. ± 0·0063(51). The possible connexion between magnesium deficiency and low fat percentages suggested by Rowallan(52) is not upheld by the investigations of Bartlett, Palmer, Rowland, Cowie & Allcroft(53,54). Archibald found that the molybdenum content of the milk of eight cows ranged from 18 to 147 μg. per l.; most of this element was concentrated in the cream fraction(55). The bromine content of seven samples of cow's milk was found by Casini to be 18·4–24·1 μg. per 100 ml.(56). The chloride content of the milk of four Friesian cows increased from 0·116% in the first month to 0·190% in the 9th to 10th month of lactation(57).

Fabris(58) determined the citric acid content of seventeen samples of milk from Friesian and Brown Swiss cows; the mean percentage was 0·254. The average histamine content of the milk of four cows has been found by Asdrubali(59) to be 0·087 μg. per ml. in early lactation and 0·053 in late lactation. In a study of ketosis in dairy cows, Breirem, Ender, Halse & Slagsvold(60) observed an increase in fat percentage with decreasing milk yield. Prevention was not achieved by feeding sugar, carotene, or compounds of P, Cu, or Co, but there was only one mild case in twelve cows fed on rations rich in cellulose and poor in sugar.

*Milk of other species*

Valen & Valen(61) report that during 1949, 119 samples of goat's milk received at various dairies in Norway gave the following average figures: fat 3·48%, protein 3·17%, lactose 4·33%, ash 0·81%, total solids 11·83%. Ulvesli, Husby & Presthegge(62) found little difference in composition between the milk of the goat and cow except in a higher fat content of the latter.

The effect of stage of lactation on goat-milk composition was investigated by Neseni & Freimuth(63); fat, solids-not-fat and casein declined during the first 11–14 weeks, but increased thereafter. Lactose was relatively constant. The yield reached a maximum of 3 kg. daily in the 3rd to 5th week. Colostrum obtained from four goats 2–6 hr. after parturition, was found by Seidler(64) to contain 14·6–31·6% total solids, 3·9–11·6% fat, 3·5–14·9% protein, 4·2–5·3% lactose and 0·8–1·3% ash. The same four goats gave milk showing the following individual average variations: 11·6–12·2% total solids, 2·65–5·20% fat, 2·29–2·77% protein, 4·06–4·74% lactose and 0·74–0·83% ash(65). Average figures for goat's milk examined in Sardinia were given by Principalle(66) as: specific gravity 1·034, fat 5·3%, solids-not-fat 9·12%, ash 0·77%, freezing-point depression 0·582° C. Milks from three mid-European breeds of goats differed in fat, solids-not-fat, protein and casein contents, but not significantly in albumin and globulin, lactose and chloride content(67). The milk of the sow was found by Heidebrecht, Macvicar, Ross & Whitchair(68) to contain 8% fat, 11% solids-not-fat, 5% protein, 0·9% ash on the 15th day of lactation. The total nitrogen of sow's colostrum was found by Sheffy, Shahani, Grummar, Phillips & Sommer(69) to be 1·646%, the concentration falling to 0·81% and 0·86% in milk drawn at the 15th and 30th day respectively. In the 7th week the casein was found to be 57·2% of the total nitrogen. The yield and composition of the milk of five New Zealand Berkshire sows was examined by Smith(70). The peak of production was observed in the 5th week and not in the 3rd as found by previous workers. The average composition of third lactation milk was: fat 7·7%, protein 6·2%, lactose 5·0%. Beacom & Bowland(71) compare the amino-acid composition of sow's milk with that of the cow and ewe. El-Sokkary, Sirry & Hassan(72) and Adam(73) publish detailed analyses of ewe's milk.

### (*b*) *Butter*

Butter made from whey cream was found by Thomsen(74) to contain less lecithin and solids-not-fat than ordinary butter. The relatively slow absorption of diacetyl by the fat phase of butter can be hastened by increase of temperature. Tuomainen(75) also found that absorption occurred more rapidly in slowly solidified than in quickly solidified butterfat. Tammisto &

Tuomainen(76) found the optimum pH for diacetyl formation during churning to be 4·0–4·3. Higher churning temperatures also favour diacetyl production. The concurrent production of methylglyoxal and diacetyl in butter starters is claimed by Evenhuis(77). Cream neutralization had no effect on the water-insoluble fatty acids or butyric acid content of the butter even when sodium hydroxide or sodium carbonate were used at 110–120° F. Over-neutralization was also without effect(78).

### (*c*) *Cheese*

Chemical, bacteriological and organoleptic characteristics of 214 samples of cottage cheese retailed in Connecticut are recorded by Morgan, Anderson, Hankin, Dowd & Foter(79). The most common 'off' flavours were 'acid', 'bitter' and 'yeasty'. An attempt to correlate cheese quality with moisture content of the fat-free sample was made by Schulz, Thomasow & Leder(80). Recommended values were 50–59% for hard cheese, 60–69% for soft and 70–82% for cream and unripened cheese. Irvine(81) found little seasonal variation in moisture and fat contents of 386 samples of Cheddar cheese made in Ontario factories during the period May to October 1950. Average values were 35·15 and 33·36% respectively. Salt varied between 0·9 and 2·9% and pH from 4·9 to 5·5. Best quality cheese had a salt content of 1·6% and a pH of 5·05–5·20. 'Fruity' and 'open' cheese had a higher pH, and cheese of low salt content often showed defects. Seasonal variations were, however, found in New Zealand Cheddar cheese by McDowall(82). Moisture content decreased to a minimum in February, after which there was a constant recovery until the end of the dairying season. The fat content remained fairly constant during the early months of the season, but showed an increase from November to February, followed by a decline. The average values for mature New Zealand Cheddar were 31·3% moisture, 37·7% fat, 25·0% protein, 6% ash, etc. By means of paper chromatography Fagen, Stine & Hussong(83) found that traces of lactose persisted in cheese for 12 days after pressing. Galactose appeared after cheddaring and persisted for 19 days in raw-milk cheese, and for 39 days in pasteurized milk cheese. Increased salt concentration prolonged these survival periods. Trautman, Hansen & Hartwell(84) conclude that the use of paraffin, pliofilm, etc., coverings does not affect the types of free amino-acids at any stage of ripening, although some differences in their rate of liberation were noticed. Kosikowsky(85) observed the presence of glutamic and aspartic acid in American Cheddar cheese a few hours after the curd was produced. Steady increases in glutamic acid, leucine, valine, phenylalanine and the basic amino-acids were shown throughout the ripening period. Fairly rapid proteolysis occurs, since at the end of 2 days at 60° F. nearly half of the total number of amino-acids had appeared. Some differences were observed between the amino-acid liberation of pasteurized as compared with raw-milk cheese(86). Asparagine appeared to be more characteristic of pasteurized milk cheese, whereas raw-milk cheese contained greater quantities of aspartic acid. Tyramine found in raw-milk cheese was not observed in pasteurized milk cheese. Wide differences in the amino-acid pattern of thirteen types of cheese foreign to America were noted by Fagen & Hussong(87). The histamine content of Finnish cheese was found to vary with individual samples of the same variety(88). Hiscox, Harrison & Wolf(89) confirmed their previous observations on the development of fat-soluble volatile acids following pricking and subsequent mould growth in Stilton cheese. The secretion of proteolytic enzymes by the mould was also apparent. In the making of Swiss cheese(90), acetic acid only was found during the early stages. In the 'hot room' (75° F.) propionic acid developed rapidly during the development of the eyes.

Losses of calcium and phosphorus via the whey are much more severe in the making of Stilton cheese (94 and 73% respectively) than in Cheddar cheese-making (43 and 50% respectively)(91). Moisture, calcium and phosphorus contents of curds were recorded by Orlova(92). The figures were: for rennet curd 69·8%, 0·40% and 0·27%; for curd obtained by calcium chloride plus heat coagulation 70·4%, 0·43% and 0·28%; and for sour curd 70·2%, 0·1% and 0·2% respectively. Losses of calcium and phosphorus in sour-milk curds were approximately 84 and 50%, and the manufacture of hard rennet cheese is, therefore, recommended by Szkilladziowa, Wojna, Rudowska & Łuczakowa(93). An extensive investigation into the moisture, calcium and

phosphorus content of many varieties of cheese was undertaken by Randoin & Jourdan-Vatinel(94,95). The Ca:P ratio had little connexion with the type of cheese. Their concentration depended on the speed of coagulation, the extent of cutting and 'cooking', and on the initial composition of the milk. The composition of fifteen Provolone and thirteen Romano commercially made cheese of ages up to 14 months was determined by Harper & Gould(96). Good Provolone cheese contained relatively large quantities of butyric acid. Acetic and propionic acids did not greatly influence the quality(97). The composition of Tilsit cheese—as modified by its mode of preparation—was investigated by Schwarz & Mumm(98). Aroma constituents of Tilsit cheese 4–4½ months old were found by Schwarz & Thomasow(99) to consist chiefly of valeric and butyric acids, ammonia and the methylamines, and methyl ketones. While the relatively non-volatile acids and bases have little influence on aroma, they may influence the taste. Defects in cheese made from milk produced on marshy land cannot be attributed to a lower calcium and phosphorus content of the milk. Schwarz, Castillo & Mumm(100) examined forty-five samples of milk produced on such land in Schleswig-Holstein and found it possessed a higher solid content, calcium, phosphorus and fat than control milk from cows on light, sandy soils. The chemical composition of some Czech and Slovakian cheese is reported by Maxa(101), and of Polish cheese by Rudowska, Szkilladziowa, Łuczakowa, Berger & Wojna(102).

## II. Chemistry of milk constituents

### (*a*) *Fat*

The de-emulsification of milk and milk products by chemical reagents was studied by Patton(103). An aqueous reagent prepared from *n*-butanol and *n*-butylamine proved to be most efficient. Fractionation of butterfat glycerides with absolute alcohol as the solvent gives thirteen fractions varying from white, hard, waxy solids melting at 54·5° C. to an orange-coloured oil freezing at −12° C. Most of the glycerides are in the mono- and di-saturated form(104). McClymont observed the uptake of acetic acid from the blood by both dry and lactating cow's udders and concluded that the short-chain fatty acid content of butterfat as indicated by its Reichert value is not directly dependent upon the uptake of acetic acid by the udder(105). No significant differences in Reichert, Polenske and refractometer values were evident between the secretions of the four quarters of the udder(106). Butterfats from Indian cows and buffaloes were fractionated by Achaya & Hilditch(107) by repeated crystallization from ether or acetone at temperatures between −50 and 0° C., and the component glycerides determined in each fraction. Tri-unsaturated and simple saturated glycerides were absent. The percentages in buffalo's and cow's butterfat respectively were: tri-saturated glycerides 39·7 and 35·1; di-saturated—mono-unsaturated 37·5 and 36·2; mono-saturated—di-unsaturated 22·8 and 28·7. The oleic acid content of butterfat was found by Bartley *et al.*(108) to vary with the iodine value, but the linoleic acid remained fairly constant. The observation was also made that during the course of lactation, the initially high iodine value fell by some 8 units in the 4th–5th month, after which it rose by about 3 units towards the end of lactation. Except in the early lactation period, when the iodine value was already high, pasture feeding increased the iodine value by about 5 units. The composition of many natural fats of animal and vegetable origin is presented by Hilditch(109). The presence of linoleic acid in New Zealand butterfat and its absence in English butterfat was noted by Shorland(110). Approximately 0·4% of New Zealand butterfat consists of $CO_{20}$ unsaturated fatty acids(111). The existence of branched-chain fatty acids in New Zealand butterfat was first noted by Hansen, Shorland & Cooke(112). Further investigation showed that they are methyl-branched-chain fatty acids isomeric with normal heptadecanoic acid, and multi-branched-chain saturated acids of empirical formula $C_{20}H_{40}O_2$ containing three or possibly four methyl groups(113). Further investigations into the seasonal variations of the fat constants of New Zealand butter reveal a close connexion between iodine values and the ratio total saturated/$C_{18}$ unsaturated acids, and between the $C_4$–$C_{12}$ saturated acids and Reichert and saponification values(114). Oleic acid content fell from 33·8% in October to 29·3% in December and then increased to 35·8% in

April. This acid is thought to be chiefly responsible for seasonal changes in iodine value(115). Hove & Harris(116) note a highly significant correlation between linolenic acid and tocopherol contents of many fats including butterfat. Cerutti(117) points out that the Polenske values of Danish butter are significantly higher than those of Italian origin. Mattsson, Thomé & Swartling(118) have tabulated analyses of butter samples for thirty Swedish creameries. They found that the $C_{18}$ conjugated and linolenic acids were higher during July and September than in January and May. Linoleic acid showed no seasonal variation. These variations are reflected in the higher iodine values of the fat of Swedish milk noticed in the summer(119). Iodine values below 30 tend to render the butter too hard, while the tendency is in the opposite direction for values above 37. By chromatographic analysis of the unsaponifiable fraction of butterfat, Morice(120) has established the presence of lanosterol previously identified with wool fat and yeasts. Four other crystalline compounds were isolated but not identified. The fat constants of sow's milk fat closely resemble those of the depot fat or lard(121), and there is evidence to support the view that the sow is more dependent than the cow upon ingested and depot fats for maintaining the milk-fat level.

### (*b*) *Nitrogenous compounds*

Difficulties experienced in the hydrolysis of protein in the presence of carbohydrates are the result of sugar amino-acid linkage. Kofrányi(122) uses 5 N-formic acid to hydrolyse starch and to dissolve proteins, leaving cellulose unchanged. The proteins are then precipitated from the filtrate and are subsequently hydrolysed with hydrochloric acid. Shahani & Sommer(123) made an examination of the nitrogen distribution of fourteen individual and fourteen mixed milk samples. While the general findings agreed with current knowledge, wide variations between individuals were observed. Laboratory pasteurization at 143° F. for 30 min. caused a coagulation of 9% of the albumin and 5% of the globulin together with a slight increase in ammonia and urea. Storage of evaporated milk at 100° F. caused an increase in non-protein nitrogen. Organic sulphur in proteins was found by Springer & Quentin(124) not to be entirely accounted for by cysteine, cystine and methionine of the acid-hydrolysate. Thus either some loss of sulphur occurs during hydrolysis or some hitherto unidentified sulphur compound is present in proteins. Block(125) has published comprehensive data regarding the amino-acid composition of the proteins of milk, cheddar cheese, dried skim milk and evaporated milk. Slight losses of lysine, histidine and arginine in evaporated milk during storage periods above 15–17 months were noted by Hodson(126). As most evaporated milk was stored for less than one year, these losses were not commercially significant. The tyrosine and tryptophane content of casein was found by Schormüller(127) to be 5·55 and 1·04% respectively; of lactalbumin 3·31 and 2·19%, of dried skim milk 1·70 and 0·81%, and of dried whole milk 2·22 and 0·58%. Lea, Rhodes & Borrell(128) observed rapid browning of a mixture of glucosamine hydrochloride and casein at pH 6·3, 70% relative humidity and 37° C., but the lysine $\epsilon$-amino group of the protein was not affected and both the aldehyde and amino groups of the substituted sugar were inactivated.

### (*c*) *Miscellaneous*

The reduction by acetaldehyde and propaldehyde of methylene blue in milk is brought about by a heat-stable constituent associated with the fat globules(129). In this and other similar reactions, ascorbic acid acts as a catalyst(130).

The well-known 'de-ionizing' effect of sodium hexametaphosphate on calcium salts is seen in the research of Barnabei & Mari(131), who found that addition of 7% to milk reduced the concentration of precipitable calcium in the trichloracetic acid filtrate from 0·140 to 0·028%. Low concentrations of hexametaphosphate had a marked delaying effect on rennet action.

### (*d*) *Enzymes*

*Phosphatase*

Morton(132) has shown that the association of enzymes with water-insoluble particles, particularly phospholipids, can be broken with ease by a simple treatment with *n*-butyl alcohol. Thus,

highly active preparations of milk phosphatase may be obtained from buttermilk by a short treatment at 35° C. and pH 5·2. Applying this procedure to skim milk, Zittle & DellaMonica (133) secured acetone-precipitated fractions which were 1000 times more active than the starting material. These authors (134) found adsorption of milk phosphatase preparations on diatomaceous silica of little value for the purification of the enzyme. They have continued their comparative studies of the alkaline phosphatase of milk and intestinal mucosa (135, 136) by determining the different degree to which the enzymes were inhibited by a number of inorganic ions and amino-acids.

Owen and his colleagues have correlated the results of measurements of the phosphatase level and of the phosphorus partition in cow's milk (137) throughout a full lactation period, and in human milk (138) for the first 4 weeks of lactation. They have studied the changes caused by bovine mastitis (139), and by the treatment of cows with thyroxine and thiouracil (140). Using phosphatase concentrates prepared from the milk of cows, buffaloes, goats and ewes, Kannan & Basu (141) have determined the rate of hydrolysis of di-sodium phenylphosphate over the temperature range 10–40° C., and the thermal destruction during holding of the samples for different times at temperatures up to 62·5° C. Hansen, Wood & Thornton (142) used a commercial H.T.S.T. plant of small capacity to study the heat-inactivation of phosphatase. Assuming instantaneous heating up and cooling, inactivation at 160° F. required 16·8 sec. Lear & Foster (143) have rightly stressed the importance of considering the effects of pre-heating as well as those of holding on the inactivation of phosphatase. Using a suitable laboratory pasteurizer which allowed straight-line pre-heating from 86° F. to the desired holding temperature in 1 min., they demonstrated that the thermal destruction during pre-heating was negligible on heating to 146·3° F., but amounted to nearly 20% of the total heat required for inactivation of the enzyme at 161·6° F.

There have not been any major developments concerning the phosphatase test. Cook & Hughes (144) have met with batches of filter-paper which caused unusually high colour readings in the Kay-Graham test, and have outlined methods by which the suitability of new batches of paper can be checked. On the basis of further experience, Wight & Tramer (145) have reaffirmed their favourable view of the suitability of the rapid test of Aschaffenburg & Mullen for the routine control of pasteurization efficiency. The test has recently been improved and simplified by the introduction of a carbonate-bicarbonate buffer (146). A new method for the preparation of di-sodium *p*-nitrophenylphosphate, the substrate used in this test, has been given by Bessey & Love (147) together with methods of assessing the purity of the reagent. Aschaffenburg (148) has suggested that final crystallization from cold rather than from hot organic solvents, and air-drying rather than desiccation should yield preparations of improved quality.

Replies from 271 laboratories in the U.S.A. and Canada to a questionnaire sent out in the spring of 1949, form the basis of a report by Burgwald (149). Of a total of 435,885 samples of milk and dairy products subjected to the phosphatase test, only 1·74% gave results indicative of faulty heat treatment. In considering this figure, and the more detailed data given in the report, it must be borne in mind that they were obtained by procedures of varying sensitivity. Thus, no less than 193 laboratories used the Scharer field test, generally lengthening the 10 min. incubation time to improve the sensitivity, and only five laboratories used the Sanders-Sager technique exclusively, despite its adoption, in 1947, as an official A.O.A.C. method. Burgwald's paper also contains original data on comparisons of the efficiency of the Scharer, Sanders-Sager and Cornell test as applied to milk and cheese.

As bovine tuberculosis is widespread in Germany, the increasing attention given to pasteurization control by the phosphatase test is to be welcomed. The value of this test has been demonstrated and stressed by Schwarz (150). He and Lange (151) as well as Kieferle & Seuss (152) have reported on the suitability of yet another modification of the phosphatase test for control purposes. This relies on the use of proprietary reagents in solid form ('Lactognost'-Heyl) which appear to be similar to those used in the Scharer test. Using the same incubation time, the sensitivity of the new modification is of the same order as that of the Scharer field test. It was

found to be markedly reduced when the test was applied to souring milk samples(153), and this may account for the rather disturbing reports(154,155) of negative phosphatase tests obtained on samples of heat-treated market milk which, according to the results of guinea-pig tests, contained tubercle bacilli. No such discrepancies were observed in an experiment by Seelemann(156) in which fresh milk infected with an allegedly heat-resistant TB-strain was used.

French workers(157) have found that the rate at which phosphatase in cream and milk is inactivated on heating to 90° C. differs but little. On further investigation of the cause of positive phosphatase tests in cream pasteurized at butter factories, they found(158) that farm-separated cream neutralized with $Mg(OH)_2$ frequently contained heat-resistant phosphatases produced by yeasts and oidia. Apparent reactivation of the phosphatase in high-temperature pasteurized cream on storage at 30 to 37° C. has been reported by Posthumus(159). However, increased colour readings were only observed when the Kay-Graham test was used, and not when the Scharer technique was applied. This seems to invalidate his speculation that some phosphatase within the fat globules remains active and diffuses into the aqueous phase on standing. The applicability of the phosphatase test to sour-milk products and cheese has been discussed by Simskaya(160,161), and the testing difficulties and limitations arising from the development of bacterial phosphatases in French cheese varieties have been considered by Camus and Alifax(162).

Confirmation of the known presence and properties of an acid milk phosphatase with optimum activity at a pH near 4·0, has come from Sweden(163) and France(164). It is of interest that the French workers found in some, but not all, milk samples evidence for the presence of yet another enzyme with optimum activity at pH 5·5–5·8.

*Rennin*

Schwander, Zahler & Nitschmann(165) have provided valuable data for crystalline rennin. From measurements of the diffusion and sedimentation constants at pH 7, they arrived at a molecular weight of about 40,000 for the main component of solutions which were not entirely homogeneous in the ultracentrifuge, though they appeared so on ionophoresis on filter paper at different pH values. The detailed amino-acid composition was determined by chromatography on starch columns. Nitschmann and his colleagues have concerned themselves with several aspects of the mechanism of rennin action on casein. They have shown(166) that proteolysis of casein is negligible at the time of clotting, when crystalline rennet is added in concentration of the order used in practice, though partial hydrolysis occurs with much higher concentrations of the enzyme. Neither the crystalline enzyme nor two commercial extracts exhibited any phosphorus-splitting action on casein(167), although the extracts showed phosphatase activity with glycerophosphate as the substrate. Elsewhere(168), an old suggestion was disproved that some of the products of the partial breakdown of renneted casein might have a clotting power of their own.

Hostettler & Rüegger(169) have dealt at great length with the effects of calcium chloride additions on rennet coagulation. According to their findings, the decrease in pH, which must be considered the primary cause for increases in the speed of clotting, is the result of interaction of calcium ions with the casein itself rather than with the phosphates of milk. Solutions of sodium caseinate prepared by acid coagulation of slow-clotting milk gave clotting times with rennet of the same order as those prepared from normal milk. It is concluded that slow-clotting is due to peculiarities in the make-up of the casein complex rather than of the casein itself. Possibly, the size of the casein particles is of major importance. As Hostettler & Imhof(170) have observed in experiments on renneting which form part of a general study by electron microscopy of casein and its reactions, the clotting time decreases as the mean size of the casein particles increases. Their bulletin contains excellent photographs of casein during and after clotting, but, as they themselves point out, no final clear-cut picture of the manner of aggregation and coagulation of the casein in milk has yet emerged from their own electron microscope findings and from those of others(171,172) who have applied this modern tool for the same purpose.

In preliminary work, McKerns(173) has suggested that paper chromatography might be of value in the purification of rennet, in amino-acid analysis of purified fractions, and in studying the break-up of the casein molecules following rennet addition. The mechanism of rennet coagulation has been discussed by D'yachenko(174) in the light of comparisons of the ability of casein and paracasein to bind acid and base. In a well-illustrated review, Schulz(175) has considered the impact of the physical and chemical changes that follow the renneting of milk on practical cheese-making problems.

Zimmerman(176) has studied the ripening of cheddar cheese made with the addition of different quantities of commercial and purified rennets. Only part of the observed proteolysis could be ascribed to the proteolytic activity of the rennet preparations.

Berridge(177) has provided a simple technique for the estimation of the clotting power of different rennets under controlled conditions. Reconstituted skim-milk powder in 0·01 M-$CaCl_2$ solution forms the substrate. A convenient device for the automatic creation of the thin film of fluid required to detect the first signs of clotting has also been described by the same author(178). For the same purpose, Titov(179) has suggested an apparatus in which 300 ml. samples of renneted milk are kept in suitable motion in a black-painted water-bath. Directions for making up a standard substrate for estimating rennet activity with dry casein as the essential ingredient can be found in a note by Salati(180).

Information concerning rennets of vegetable origin is given in (181,182,183).

*Other enzymes*

Herrington(184) has reviewed present-day knowledge of lipase, the flavour defects caused by the enzyme, and preventative measures. He expresses a firm belief that milk lipase is not a single enzyme, but a mixture of enzymes, and he is, therefore, critical of the use of specific substrates, such as tributyrin which continues to be favoured. Thus, Kannan & Basu(185) have used it in studies of the milk of cows, buffaloes, goats and sheep in relation to stage of lactation, season and other factors. Canadian workers, too,(186) have accepted it in developing a rapid technique modified from that of Dunkley, which they have applied to cheese(187) in connexion with studies of the lipolytic activity of bacteria. Loncin & Jacqmain(188) have concerned themselves with the lipase of human milk. Agitation was found to lead to increases in titratable acidity much more drastic than those observed with cow's milk.

Polis & Shmukler(189) have given details of a method of preparing crystalline lactoperoxidase by displacement chromatography of crude fractions obtained by salting-out from the rennet whey of skim milk. Unlike earlier methods, the new procedure did not fail at certain seasons. The molecular weight (82,000), determined by light scattering, agreed well with that calculated for 1 atom Fe per molecule (81,000). Electrophoresis revealed the presence of two components. Chance(190) has continued his studies of the mechanism of the lactoperoxidase-$H_2O_2$ reaction, which is shown to be the same as that of horseradish peroxidase, but to differ in velocity. From Germany, where heat treatment of milk severe enough to inactivate the peroxidase is still widely applied, have come several contributions(150,191,192,193) concerning the technique and application of the peroxidase test. Buruiana(194) has prepared concentrates of Schardinger's enzyme by repeated centrifuging and washing of cream, and has studied the kinetics of the action of this enzyme with the aid of eH-measurements. With Geraniou(195), he has given a method of estimation based on the reduction of nitrate in the presence of benzaldehyde. This method was sufficiently sensitive to establish the presence of some Schardinger enzyme in the majority of human milks, but the level was found to be so low that addition of 5% of cow's milk could be detected. Human colostrum contained more of the enzyme than later milk.

Several studies of the xanthine oxidase and xanthopterin oxidase of milk have led to the conclusion that they are likely to be one and the same enzyme(196,197,198). Harris & Hellerman(199) have pointed out that xanthine oxidase has many of the characteristics of a sulphydryl-enzyme. Polonovski and his co-workers have continued their studies of the enzyme (200,201), and have obtained good yields by the treatment of cream with dimethoxy methane(202).

The presence of aldolase in cow's milk has been established by Polis & Schmukler(203), the concentration being of the same order as in blood serum. The enzyme may be concentrated by low-temperature fractionation with ammonium sulphate. It is readily inactivated by milk acidification or by heating for 20 min. at 45° C. Human colostrum has been found to contain a proteolytic enzyme with cathepsin-like activity(204). The crystallizable trypsin inhibitor found in relatively high potency in cow's colostrum has been further investigated in greater detail(205,206). The presence of the inhibitor may be of considerable importance to the new-born calf, as it may prevent the tryptic digestion of the ingested colostral immune-globulins before their transfer to the blood stream.

Lembke & Christiansen(207) have studied the effect of irradiation with ultra-violet light of 2537 A. wave-length on a number of milk enzymes. Catalase, phosphatase and lipase were partially inactivated; Schardinger's enzyme and protease remained unimpaired. That the phosphatase activity of milk and milk products is only slightly affected by $\gamma$-radiation has been reported from the United States(208). Changes in the activity of lipase, protease and phosphatase during the ripening of Cheddar cheese have been studied by Kannan & Basu(209). Information about the effect of preservatives on the phosphatase and peroxidase test can be found in a paper by Paschke(210).

## III. Physical chemistry

### (*a*) *Proteins*

*General*

As electrophoretic studies have proved of great value in the examination of systems containing a mixture of proteins, such as blood serum, it is not surprising to find an increasing number of attempts(211,212,213,214,215,216) to apply the technique to the examination of skim milk. The published evidence suggests that elucidation of the properties and behaviour of the milk proteins by this technique presents formidable problems. Thus, great care seems required in the necessary clarification of the opaque skim milk by dialysis(211), and in the choice of a suitable pH for measurement, since the percentage distribution and even the number of the protein components vary with changes in pH(211,213). It is interesting to note that Slatter & van Winkle(213) obtained the best resolution at pH 6·6, i.e. at the 'natural' pH of milk. That identification of the components presents difficulties is indicated by the fact that numbers or letters, rather than names, have been used in referring to the components. Changes caused by heating(212,213) and centrifuging(214) as well as differences between colostrum(215) or late lactation milk(216) and full lactation milk have, so far, been studied by electrophoretic examination.

Highly promising results have recently been obtained by the use of another modern technique. By following the fate of $C^{14}$-labelled amino-acids injected into the blood streams of lactating animals, strong evidence has been obtained that casein(217,218,219) and whey proteins(218,219) of milk are largely synthesized from the free amino-acids of the blood rather than from those incorporated into the plasma proteins. Biological assays of the percentages of 16 amino-acids in two preparations of isolated fat globule membrane protein have shown(220) that the composition of this protein differs markedly from that of the major milk proteins.

*Casein*

The outstanding development in recent research on casein has been the introduction of electron microscopy by Nitschmann(221). Progress in optical resolution is strikingly demonstrated by the picture of casein particles shown in his paper. Nitschmann's work was quickly followed by a detailed study in which Hostettler & Imhof(170) have demonstrated the potential power of this research method by work comprising not only the examination of milk and of a number of milk products, but also of the changes caused by acid and rennet coagulation, heating, etc. Space does not permit a full discussion of their interesting observations which should be consulted in the original. The electron-microscope work strengthens the view that the casein

particles in milk are spherical. Most of the particles were found to have a diameter of 50–100 mμ. In fair agreement with these findings, Russian workers (222) have found an average diameter of about 100 mμ from light-scattering measurements. They used the same technique (223) to calculate molecular weights for α-casein solutions that had been subjected to rather drastic chemical treatments.

McMeekin and his associates have continued their studies of casein. Utilizing the known data for the complete amino-acid composition of α- and β-casein, they have calculated values for the specific volume which agree closely with those they derived from density determinations (224,225). They have described a method for the isolation of γ-casein (226), and, later, two procedures for the separation of α-, β- and γ-casein (227). The possibilities of separating these fractions by paper electrophoresis are indicated in a recent brief communication by Zweig & Block (228). According to Hipp, Groves, Custer & McMeekin (226), γ-casein, characterized by a very low phosphorus content (0·11 %), has an iso-electric point of pH 5·8–6·0, and accounts for about 3 % of the total casein. The results of fractionation experiments by Cherbuliez & Baudet (229) differ in some respects from those of the American workers. The fraction to which they apply the term γ-casein amounts to about 10 % of the total casein, and its iso-electric point is given as pH 5·2. About 5 % of a fourth fraction, δ-casein, was found to be present; probably this is identical with Hammarsten's proteose. In further work, Cherbuliez & Baudet (230) have studied the action of rennin, pepsin, chymotrypsin and papain on casein and its fractions. Nitschmann & Zürcher (231) have demonstrated by electrophoresis experiments that α- and β-casein tend to form complexes to a degree depending on the protein concentration. Dovey & Campbell (232) have drawn attention to marked species differences: rabbit casein was found to contain more α- and less β-casein than that of the cow, and goat casein contained almost equal proportions of the two fractions. Kamal & Turner (233) have studied the effect of iodination on the electrophoretic pattern of casein. It leads to the disappearance of the separate α- and β-peaks under formation of a single homogeneous component.

Ter Horst (234) has made an analytical study of fractions of calcium caseinate sols prepared by super-centrifugation (not ultracentrifugation, as stated in her paper), and draws attention to the formidable difficulties that arise in attempting a sound interpretation of results obtained in this manner. On the basis of viscosity studies, Higgins & Hayes (235) have discussed the changes in the shape of casein particles that occur after acidification, renneting or treatment with alkali. Data on the swelling of casein granules in the presence of a variety of swelling agents can be found in (236). Kiermeier (237) has shown that ion-exchange reactions occur when calcium salts are allowed to react with suspensions of casein and para-casein, and that the results can be expressed in terms of adsorption isotherms. The coagulation of casein by heat, and the effects of added calcium salts in promoting this reaction, have been studied (238,239), and attention has been given to the formation of gels in alkaline casein solutions (240,241,242), with particular reference to the role of the sulphydryl groups. Mellon and his colleagues have continued their investigations of the mechanism of water absorption by casein, and of the groups involved in this reaction (243,244,345).

A kinetic study of the digestion of casein by trypsin has been presented by Fraser & Powell (246). The casein behaved as a two-component system, the smaller component (β-casein?) reacting much faster than the larger one. A striking example of the difference between casein fractions in their interaction with enzymes has been given by Perlmann (247), who found that prostate phosphatase dephosphorylated α-casein to a marked degree, but did not affect β-casein. In fact, β-casein inhibited the reaction with α-casein. Methods have been given for the hydrolysis of casein with $SO_2$ (248) and cation exchange resins (249), and for the catalysis of acid hydrolysis by titanous and stannous chloride (250).

Pedersen (251) has given a detailed report of the factors that are of importance in the manufacture of high-quality rennet and acid casein. Information about the plants and processes used in the manufacture of casein, and other milk products, is to be found in a well-illustrated paper by Kastens & Baldauski (252).

Studies of the ripening and flavour characteristics of cheese are likely to benefit from the use

of paper chromatography, a technique which, as shown by recent publications(253,254), permits the determination of the amino-acid pattern of almost any type of cheese with comparative ease. Flavour and aroma may depend on the distribution of the various amino-acids rather than on the presence of any particular amino-acid(254). Even more important in this respect may be the intermediate breakdown products of casein, as pointed out recently by Storgårds(255), who has examined the electrophoretic patterns of several types of cheese which he found to differ markedly in the number and distribution of polypeptides.

*Whey proteins*

A wealth of information is to be found in the recently published thesis of Wegelin(256), which, in addition to a detailed review of the literature, contains the results of numerous analytical and electrophoretical experiments with acid and rennet whey. The distribution and nature of the proteinaceous components are discussed, as are the changes caused by processing, heating in particular. The results of animal feeding experiments with dried whey prepared by electrodialysis are also reported. Electrophoretic comparisons have been made between the protein make-up of whey from the milk of the cow and the human. Results of such comparisons are also to be found in (257).

Stanley, Whitnah & Andrews(258) have made a critical examination of the effects of the method of preparation on the electrophoretic pattern of whey proteins, pointing out the difficulties of analysing the different patterns obtained after acidification, renneting and salting-out of milk, difficulties which must be added to those known to arise with any given preparation from variations of the pH of buffering.

From a brief report by Hellbrügge(259), it appears that the electrophoretic pattern of the whey proteins is not affected by brief irradiation with ultra-violet light, but that changes suggesting denaturation occur on irradiation for up to 1 hr.

It now seems almost certain(260) that bovine whey contains a small amount of blood-serum albumin. The product, isolated in the crystalline state from milk, had the same physico-chemical properties as serum albumin except for a slight difference in its electrophoretic behaviour. Serological identity was obtained in experiments in which the sensitive Schultz-Dale technique was used(261). Gordon & Semmet(262) have made a thorough study of Sörensen's 'crystalline insoluble substance' for which they suggest the name α-lactalbumin. This component, characterized by insolubility in water and dilute salt solutions, has a molecular weight of about 16,000, and contributes about 12% to the total whey proteins. Searching for the factor responsible for the development of 'sunshine' flavour in homogenized milk, a team of workers at East Lansing(263,264) have isolated a minor whey-protein fraction which appears to be the agent responsible for the development of this flavour defect. The composition and properties of the fraction have been investigated. Though prepared by similar methods, it differed from the σ-proteose described by Aschaffenburg. Data on the degree of denaturation of the soluble proteins of milk resulting from heating to 145–175° F. for varying periods, are to be found in a paper by Harland, Coulter & Jenness(265), in which they suggest utilization of this criterion for the control of the manufacture of 'low-heat' skim-milk powder. Applying an argentometric-amperometric titration method to skim milk, whey and its fractions, Hutton & Patton(266) have shown that β-lactoglobulin is the main carrier of sulphydryl groups, and that it is the presence of this component that gives rise to the development of 'cooked' flavour on heating. Johnson & Swanson(267) have described a quantitative method of determining the concentration of whey proteins by the biuret reaction. Unfortunately, it requires preparation of the samples by dialysis. Following earlier work by Heinemann and his associates on the determination of milk fat by area measurements of spread monolayers, this author and Parker(268) have now demonstrated that the same principle can be used for the determination of the protein content of whey.

*β-Lactoglobulin.* From crystallographic data obtained by electron microscopy(269) and X-ray analysis(270), a molecular weight of 35,800 has been calculated for anhydrous crystals of this protein. A value of 35,700 has been reported(271) as the result of light-scattering measurements.

Information on the shape and degree of hydration of β-lactoglobulin in solution is to be found in an investigation (272) of a number of proteins by the method of small-angle scattering of X-rays. Further evidence of the electrophoretic heterogeneity of crystalline β-lactoglobulin has been presented by Jacobsen (273) and by Polis, Schmukler, Custer & McMeekin (274) who have proceeded to separate the slow-moving fraction of 40% from the fast-moving component of 60%. The isolated fraction was electrophoretically homogeneous, its iso-electric point was pH 5·3, and its molecular weight 36,100. As with casein, McMeekin & Marshall (225) obtained good agreement between the values for the specific volume of β-lactoglobulin calculated from the amino-acid composition and those determined by density measurements. Evidence for the direct combination of salts with proteins has been obtained in experiments on the uptake of salts by large single crystals of β-lactoglobulin (275). The preparation and properties of the crystalline compound of the protein with dodecyl sulphate have now been described in full (276), and the mechanism of the denaturation of the compound and of β-lactoglobulin itself in alkaline solutions has been studied (277). The rate of reaction which rises rapidly from pH 8 to 9·5, was found to be faster for the pure protein, and the same at 3 and 25° C., whilst the compound showed the more usual increase in rate with rising temperature. Weil & Buchert (278) have made a detailed study of the photo-oxidation of β-lactoglobulin in solutions containing methylene blue.

Larson & Jenness (279) have found that most of the reagents normally used fail to react with the sulphydryl groups of crystalline β-lactoglobulin in solution. Greater reactivity is obtained on heat-denaturation which, over the temperature range 64–75° C., has been shown to proceed in accordance with the kinetics of a first-order reaction. Further data on the amino-acid composition of β-lactoglobulin, determined by paper chromatography, are to be found in (280).

*Immune proteins*

McDougall (281) has presented the results of a study of bovine pre-colostrum, the udder secretion obtainable before parturition, dealing in particular with the characteristics of the immune-globulin fraction separated by ammonium sulphate precipitation. Smith & Brown (282) have used the ultracentrifuge to compare the immune proteins of cow's colostrum and milk with globulin fractions isolated from blood plasma. Great similarities were found, the major component with a sedimentation constant of about 7S always being accompanied by a component with a constant of about 10S. The authors point out that the molecular weight of the heavier component is approximately double that of the 7S-component of 180,000. The marked protective value of a single feed of about 14 g. of colostral immune-globulins has been demonstrated in experiments with newborn calves (283). Electrophoretic patterns of whey prepared from sow's milk at all stages of lactation are to be found in (284). The seventh paper in a series on the properties of the colostrum of the dairy cow by workers from Manhattan, Kansas (285), deals with the pH, buffer capacity and osmotic pressure during the first fortnight after calving. Collodion bags were used in determining the osmotic pressure, and it is claimed that this fell by as much as one-third during the transition from colostrum to milk. As the spread of values for milks tested on the 14th day after calving, i.e. at a time when normal milk is being secreted, far exceeds the established range of variations, this claim needs substantiation by a proven method of determining osmotic pressure.

### (*b*) *Other aspects of physical chemistry*

*Fat*

Several papers concerning the size-frequency distribution of the fat globules in milk have come to hand. Schmid (286) has given a detailed description of an accurate and simple technique for counting and measuring fat globules. Odenwald (287) has studied the effect of state of lactation, feeding and climate on fat-globule size. Characteristic breed differences were found. Data on Russian breeds of cattle are to be found in (288). The effects of souring have been studied by Indian workers (289), whose paper contains results obtained with buffalo milk. A technique for determining the size-frequency distribution of the fat globules in sow's milk has been described

by Whittlestone(290), who found no systematic trends in distribution when examining samples drawn at different stages of the milking-out process. Nor were any such trends apparent during the lactation period(291) excepting the colostral period, when a narrow size-distribution was found. A method for measuring the fat globules in homogenized milk has been described(292). Ashworth(293) assesses the efficiency of homogenization by photoelectric measurement of the turbidity of samples 'cleared' with ammonia, and diluted with hot water. The method is also applicable to skim milk and milk powder. King has presented reviews on the effects of agitation on the fat in milk and cream(294), and on the structure of the fat globules in butter(295), and has studied the structure of Alfa butter(296). Using a dilatometric technique, Mohr & Kaufmann(297) have established the relationship between specific volume of fat and temperature for milk, butter-milk, butter and creams of varying fat content. A calorimetric study of cream rapidly cooled from 30° C. to lower temperatures is due to Rishoi(298).

*Freezing and frozen storage*

Nickerson, Coulter & Jenness(299) have studied the process of freeze-drying as a possible alternative to spray-drying. Partial destablization of the milk fat proved to be the main drawback. This could be reduced by homogenization, but not sufficiently to prevent difficulties on reconstitution. Flavours and keeping properties were essentially similar to those of spray-dried products. Davidov(300) has described a process of concentrating milk two to three times with relatively small losses in solids by partial freezing at $-1{\cdot}2$ to $-2{\cdot}1$° C. A series of studies by Italian authors(301,302,303,304) indicates that prolonged storage of milk at $-25$ or $-40$° C. does not cause any major changes in its physical and chemical properties. The casein complex of the frozen whole milk remained stable for 180 days, a finding in contrast to that of Babcock, Strobel, Yager & Windham(305), who, working with homogenized milk, report noticeable casein flocculation after 100 days' storage at $-23{\cdot}3$° C. Addition of sucrose, or sucrose and ascorbic acid, improved the keeping properties. Improvements have also been found(306) to result from partial removal of the cations, particularly those of calcium, by ion-exchange treatment of the milk, or from heat treatment at 77° C. before homogenization(307).

Mucha & Bell(308) have studied the keeping properties of products prepared by separating, pasteurizing and homogenizing cream and recombining it with the pasteurized separated milk. The elaborate process seems hardly worth while, as no advantages emerged apart from a reduced tendency to develop oxidized flavour defects. Problems arising in the frozen storage of concentrated milk have been discussed by Doan(309), and further investigated by Wildasin & Doan(310), and by Bell & Mucha(311). Wildasin & Doan observed increased protein instability as the result of homogenization, and found any delay in freezing after concentration of the milk deleterious. According to Grant(312), goat's milk retains its stability and flavour for at least 5 months on storage at $-17$° F. No advantages resulted from homogenization. Hard Dutch cheese of satisfactory quality has been made in Russia(313) from milk that had been kept in frozen storage. Useful information concerning the refrigerated storage of cheese is to be found in(314). Freezing-point values for different types of cheese are listed as an aid in selecting storage temperatures at which actual freezing of a particular type is avoided.

*Miscellaneous*

Indian workers have continued their studies of the refractive index of milk and of factors affecting it(315,316). An average of 7·26 has been reported for the pH of human milk drawn and tested without exposure to air(317). The relationship between pH and the titratable acidity of milk has been discussed at great length by Roeder(318,319), and the shortcomings of titratable acidity determinations during the initial stages of cheese-making have been pointed out by Hansson(320), who suggests electrometric methods for greater accuracy. The theoretical and practical aspects of measuring oxidation-reduction potentials in general and more specifically in milk are dealt with by Krauss(321).

Much information on the conductivity and chloride content of human milk is to be found in (322,323,324). Further attention has been given to the process of electrodialysis. Wegelin(256) has shown that the electrophoretic protein pattern remains largely unchanged when whey is electrodialysed. The technicalities of the process have been investigated by Mohr & Corte(325), who applied it to skim milk(326) as well as to whey. As a means of producing sterile whole milk, it has been found unsuitable(327).

Values for the thermal conductivity of whole milk, skim milk, buttermilk, whey and evaporated milk have been tabulated by Riedel(328). 'Rough' flavours noted in tasting heated milk have been shown(329) to originate from heat-coagulable whey constituents. Whey is also the major contributor to the ammonia production that occurs on heating milk in open or sealed containers(330). Patton has continued his studies of heated milk, reporting on the mechanism of formation of furan compounds(331) and the browning of heated skim milk and of evaporated milk(332). Browning during the sterilization and storage of evaporated milk has also been investigated by Tarassuk & Simonson(333), who found it to be closely related to the $CO_2$ tension in the cans. The production of fluorescent compounds was observed to be favoured by less severe heat treatment than was required for browning. That browning leads to losses in the amino-nitrogen content of spray-dried whole milk has been found by Moster & Chapman(334). Lea and his colleagues have devoted much time and effort to the elucidation of the complex interaction of amino-acids and sugars(335,336,337,338,339,340,341), of which browning induced by heat-treatment is but one manifestation. Lea himself has reviewed the subject(342).

Brunner(343) has tested a number of agents for their efficacy in preventing foaming of skim milk and whey during vacuum condensation. Water-insoluble materials were most effective. Panasenkov(344) has measured the size distribution of the particles in commercially dried whole and skim milks, and Janzen & Swanson(345) have observed that particles of dried skim-milk powder of intermediate size are more soluble than finer or coarser particles. According to Hollender(346) the solubility of dried whole milk can be improved by incorporation of various surface-active agents. The undesirable occurrence of crystals of calcium citrate in skim-milk powder(347), evaporated milk(348) and sweetened condensed milk(349) has been considered. Bergum(350) has made a detailed study of lactose crystallization in different types of whey cheese. The different X-ray diffraction patterns of $\alpha$- and $\beta$-lactose are said(351) to allow the determination of the two forms of the sugar in dried skim milk and sweetened condensed milk. Methods for the production of lactose of a high degree of purity by treatment of whey with ion-exchange resins have been described by McGlasson & Boyd(352).

## IV. Defects of milk and milk products

### (*a*) *Adulteration and its detection*

*Added water.*

*The freezing-point test.* There have been few further contributions concerning this important test. Arrington & Krienke(353) have given the results of freezing-point determinations for sixty-four samples of genuine milk. Indian workers(354) have obtained average values of $-0{\cdot}548°$ C. for cow's milk and $-0{\cdot}552°$ C. for the milk of the buffalo, values based on the analysis of 1300 samples. The authors have found mercuric chloride superior to ten other preservatives(353). Rees(356) has determined the freezing-point and composition of the milk of a single cow throughout a lactation. As in his earlier work on Tasmanian bulk milk, he obtained an unusually high correlation between freezing-point and non-fatty solids. Lythgoe(357) has subjected original data and literature values for genuine milk samples to statistical treatment. His conclusions are open to question, as the assumption of a normal Gaussian distribution for freezing-point values does not hold. The same author(358) has given further consideration to the possibility of correcting the freezing-point for developed acidity.

The results of freezing-point tests for 1450 samples of New York City market milk have been given by Paley & Tzall(359). Values between −0·530 and −0·535° C. were most frequent, and the authors argue that the shift from the accepted mean of −0·550° C. might be ascribed to improved methods of production and handling, resulting, for instance, in a lowering of the acidity. Based, as they are, on analyses of samples of unknown purity, the arguments would seem to carry little weight.

Attention has been drawn by Rothman(360) to the considerable danger of adulteration during the pasteurization of milk in H.T.S.T. plants.

*Other methods.* According to Ystgaard, Homeyer & Bird(361), lactometer readings form a suitable basis for a sorting test for watered milk. The authors have made a statistical study of the most reliable formulae for the calculation of added water from lactometer readings or the freezing-point value. An adverse report on the use of milk-serum refractometry for the detection of extraneous water has been presented by Hoffmann(362). Sager(363) has suggested refractometry of human milk after ultrafiltration; 5% of added water can be detected in this way. Measurement of surface tension(364,365), viscosity(365) and buffering power(366) has also been suggested as means of detecting extraneous water.

*Other types of adulteration*

According to Stoldt(367) determination of the ash and of the refractive index of a mercury serum will serve to detect adulteration of human milk with cow's milk or water. The presence of cow's milk in human milk can be demonstrated by the formation of a coagulum after boiling with a cadmium sulphate solution(368). Methods based on casein determination have been recommended to detect whey additions to cow's milk(369). In Norway, where cow's milk is cheaper than goat's milk, serological techniques have been used with success to detect the presence of even small amounts of cow's milk in goat's milk(370). Butter adulteration has been the concern of Italian workers(371,372,373,374).

### (*b*) *Flavour defects of milk and milk products*

The problem of oxidative deterioration and of its prevention has again received much attention. Reviews have dealt with oxidized flavour in liquid milk(375) with the relationship between carotene and tocopherol content and the ability of milk to resist oxidation(376), and with antioxidants(377,378,379).

The influence of the type of feed on the development of flavour defects in milk has been studied by several authors. Krukovsky(380) has found milk from cows on orchard grass more resistant to oxidation than that from cows on other kinds of pasture. No marked differences were observed by Norwegian workers(381) in comparisons in which large quantities of grass-clover-hay and the corresponding silage were fed to a group of cows. The tendency to develop oxidized flavour was not enhanced by feeding herring meal, but did increase on cod-liver oil(382). Addition of copper to the diet of cows for 6 days had no adverse effect on the milk produced by the animals(383).

Recent detailed researches into the nature and origin of the oxidized flavour of whole milk by Pont(384) and Krukovsky(385) have served to emphasize the complexities of the problem. They show clearly that oxidized flavour is made up of a 'cardboard'(384) or 'chalky' or 'soapy'(385) flavour associated with the skim milk and an 'oily-metallic'(384) or 'metallic-fishy'(385) component associated with the butterfat. According to Krukovsky(385), destruction of the fat-globule membrane reduces the susceptibility of milk. The ability of manganese to counteract the deleterious effect of copper has been demonstrated by Forster & Sommer(386). The development of oxidized flavour in milk containing added copper is not affected when the milk is exposed to a direct current flowing between electrodes made of various metals(387).

Mention has already been made(263,264) of the isolation of the whey fraction said to be responsible for the flavour defect observed on exposure of homogenized milk to sunlight. Other papers in the same series deal with the susceptibility of the milk of individual cows(388) and with a number of factors that promote or prevent this flavour defect(389). Weinstein(390) has observed that cow's milk is much more prone to develop copper-induced oxidative faults than human milk. Propylgallate has been found highly effective as an anti-oxidant for milk stored at 35° F. for 14 days(391). Several anti-oxidants have been tested and found satisfactory for cream kept for long periods in frozen storage(392). Ascorbic acid additions (50–100 mg. per kg. cream) appear to be highly efficient for this purpose(393), provided the cream is not contaminated with copper. No benefit was derived from additions of α-tocopherol. Suitable methods of processing cream designated for long-time storage at low temperatures have been discussed by Olson(394).

Keeney & Doan(395) have made a detailed investigation of the fractions obtained by vacuum distillation of oxidized butterfat which had the organoleptic properties typical of oxidized flavour defects. They were components of the volatile neutral fraction, and of an unsaturated ketonic nature. Compounds of a similar α-, β-unsaturated structure have likewise been isolated by Holm, Wode & Thomé(396), who applied the technique of molecular distillation to eluates from $Al_2O_3$-adsorption columns. High negative correlation was obtained between the concentration of these compounds and the flavour score of stored butter. In earlier work from the same laboratory(397), the effect of linseed or soya-bean supplementation of the winter rations of cows on the production of poly-unsaturated fatty acids in butter prepared from the milk of these cows has been studied. Feeding of linseed oil leads to increases in the concentration of conjugated $C_{18}$-acids.

In a series of studies in which the acid, Kreis and peroxide values were widely used, Mukherjee and his associates have evaluated many anti-oxidants for butterfat(398,399), and Mukherjee himself(400) has given attention to a variety of factors that influence the development of flavour defects. Such defects in Quebec butter have been the concern of Canadian workers(401,402,403) who have given particular attention to the deleterious effects of copper and iron contamination. The effect of these metals on stored butter has also been the subject of further work by Barnicoat(404). Elsewhere(405), this author has looked into various possibilities of predicting the storage life of butter from analytical data or from the results of accelerated storage tests. Determination of the aldehyde value of butter that had been held for 4 hr. at 110° C. gave some measure of success. The effect of copper, iron and manganese on the flavour and keeping properties of butter has been studied by Dutch workers(406), and that of iron in particular by Ruuber(407).

Problems concerning dried whole milk have been investigated in a number of laboratories. Results obtained by Coulter, Jenness & Crowe(408) confirm the deleterious effects of high moisture content, and of high temperature storage on the flavour of spray-dried milk packed in air or nitrogen. In this, as in later work from the same laboratory, particular attention has been given to changes in the various reducing systems. In the later work(409), changes in the concentration of thiamine disulphide-reducing substances reflected flavour deterioration of dried milk over a storage period of 10 weeks at 37° C. when the milk had been spray-dried after preheating to about 95° C. for about 60 sec., but not with milk pre-heated at lower temperatures for much longer times. That pre-heating to as high a temperature as 110° C. has no advantages over treatment at about 90° C. has been shown by a team of British workers(410). Attention has been drawn to the beneficial effect of deaeration of whole milk before heat treatment(411,409), a procedure that decreases the oxidation-reduction potential and improves the keeping properties of the dried product. Ashworth and his co-workers have presented a series of papers dealing with the influence of various methods of pre-heating and concentration before spray-drying on the keeping properties of the resulting products(412,413), the value of the peroxide test as an indicator of oxidized flavour development(414), and improvements attainable through the addition of a mixture of ascorbic acid and sodium citrate, but not by the addition of either reagent by itself(415). The authors(416) have reported favourably on the anti-oxidant properties

of a series of polyhydric alcohol esters of stearic acid of the type used as emulsifying agents in the manufacture of ice cream, and they have also obtained good results with nordihydroguaiaretic acid, an antioxidant which, according to Stull, Herreid & Tracy(417), is particularly effective in retarding the development of oxidized flavour when a small amount of added citric acid is also present in the spray-dried product.

Kaegi(418,419) has given adverse reports on the feeding of silage to cows, particularly before or during milking, because of the ensuing undesirable odour and flavour of milk. Following earlier reports on the tainting of milk, cream and butter caused through the ingestion of *Coronopus didymus* by the cow, Forss(420) has found by analysis of the essential oil of the plant that benzylmercaptan is the responsible agent. Two weeds have been identified as the source of 'skunkweed' flavour in Oklahoma butter(421).

## V. Analysis of milk and milk products

### (*a*) *Milk*

The problem of obtaining representative samples from weigh tanks is discussed by Schwarzkopf(422), and Forschner(423) describes an automatic sampling device which collects 0·2% of the milk as received in the collecting tank. The preservation of samples for analysis has received considerable attention. 'Orthomercurate' tablets, consisting of 20% sodium orthomercurophenylcarboxylate, 20% potassium dichromate, 60% sodium chloride and a trace of fuchsine have been tested in New York State. Whilst being good preservatives, their possible toxicity has yet to be examined(424). The addition of sodium carbonate and hydrogen peroxide to milk, while showing satisfactory preserving qualities, had an adverse effect on colour and taste(425). An increase in soluble nitrogen at the expense of casein nitrogen occurred when 0·1% of 130 vol. hydrogen peroxide was added to milk(426). Voiret(427) claims that milk will keep indefinitely by addition of 0·5–1·0% of a saturated solution of salicylic acid in amyl alcohol, followed by 6–12 hr. incubation at 55–70° C. The efficacy of various antibiotic preparations in the preservation of milk has been studied by Greene & Bell(428). Bindewald(429) used a concentration of 20 p.p.m. of streptomycin which proved an efficient preservative for human milk. No adverse affects of this milk on infants were observed during a three-year trial. Milk preserved with citric acid frequently caused disorders amongst young infants. Added formaldehyde may be detected by adding milk to an equal volume of Nessler's reagent. A black ring of precipitated metallic mercury forms at the junction of the two liquids(430). The British Standards Institution have issued specifications of apparatus and methods of procedure for milk analysis—including such well-tried methods as the Röse-Gottlieb and Kjeldahl(431).

Acid-less rapid methods for estimating fat in milk continue to receive attention. Schain's(432) single-solution detergent method, with suitable corrections, gives results for fat percentage which compare well with the Röse-Gottlieb estimation. A number of anomalies in the method were observed by Sager, Grant & Hammond(433). In a method described by Tsvang & Litvinenko(434) the milk is treated with an equal volume of 10% sodium carbonate solution and a mixture of one part amyl alcohol with 6 parts ethyl alcohol. Agreement with the Gerber method was given by 464 of the 560 analyses, the remainder deviating 0·1–0·3%. Gentilini(435) uses 11 ml. of 60% sodium salicylate, 1 ml. of isoamylalcohol and 10 ml. of milk. After shaking vigorously, the tubes are placed in a water-bath at 65° C. for 5 min., centrifuged for 5 min., and replaced in the water-bath before taking the reading. Two optical methods have been described, one refractometric(436), and the other based on the close relationship between degree of transparency and fat concentration(437). Calibration of Gerber butyrometers by means of an automatic mercury pipette controlled by an electromagnetic device which prevents variations of more than a few milligrammes between deliveries, is described by de Haan & Eisses(438). A device for reading thirty-two Gerber tubes while still immersed in the water-bath is detailed by Posthumus(439). New specifications for the Gerber test are published in the Netherlands Standard Specification N. 962(440). Hingst & Thomasow(441) find that physical properties are no criteria of suitability of

amyl alcohol for the Gerber test. It should be tested either by 'blank' determinations substituting water for milk, or by tests on milk of known fat content. Gravimetric determination by the Weibull-Stoldt method is dealt with by Stoldt(442,443), and Mohr & Koenen(444). Since fat extraction from dairy products is hampered by the presence of moisture, Anselmi & Cesari(445) recommend dehydration with anhydrous sodium sulphate before extraction. Proposed alterations in the Babcock test(446) involve pipette capacity and the use of a coloured mineral oil to eliminate the top meniscus and thereby improve precision of reading. In a modified Babcock test(447), sulphuric acid is replaced by a non-ionic detergent and a phosphate salt, and enough 50% methanol is added to bring the liquids into the graduated neck of the flask. For homogenized milk(448), an improvement is achieved by adding alkyl dimethyl benzyl ammonium chloride to the sulphuric acid (sp. gr. 1·820 at 68° F.), and by using the coloured oil to remove the upper meniscus. Hem & Døvle(449) describe the Lindstrøm method, and compare its accuracy with the Gerber and Röse-Gottlieb procedures.

Storage of cream for 4–6 days at 20° C. was found by Lehmann(450) to result in decreased fat percentages as determined by the Röse-Gottlieb method, whereas by the Gerber procedure it gave increased values. The author considers that fat decomposition and poor miscibility of the sour cream were mainly responsible. Addition of 0·1% potassium dichromate and storage at 4° C. is recommended for samples destined for analysis. Calculation of the fat percentage of cream from the formula $F = 100 - (1{\cdot}1 \times \%$ moisture) has been found(451) to give good agreement with butyrometric techniques except when the samples contained extraneous water. Schulz, Effern & Roland(452) have modified the Gerber technique for use with sweetened dairy products. An early indication of fat oxidation is given by the shape of the infra-red absorption curve of the steam vaccum distillate of milk fat in the region of 5·5–6·2 $\mu$(453). Dunkley(454) finds that 2-thiobarbituric acid heated with acidified milk develops a red colour which is dependent on fat oxidation and which is well correlated with flavour defects. This reaction has been further examined by Patton & Kurtz(455). Of numerous pure compounds tested, only malonic dialdehyde, its diacetal, and glyoxal gave a similar colour, the former being spectrophotometrically identical with the colour developed from oxidized fat. Since only part of the colour-forming material of oxidized fat can be extracted with water, it is suggested that a water-insoluble precursor of malonic dialdehyde is also involved. Full details of procedure and necessary precautions are given by Dunkley & Jennings(456). Limitations of the accepted methods for the determination of lecithin have been reviewed by Kieferle, Feichtner & Pirner(457), who observe that the high values obtained for buttermilk from the Fritz process are associated with its higher fat content.

The use of the Dietert speed oven for moisture determinations is described fully by Doan(458). Ystgaard, Homeyer & Bird(459) compared the Brabender with the Mojonnier moisture tester, and found that on homogenized milks the Brabender method gave the greater conformity of duplicates, but the mean values were 0·19% lower than those obtained by the A.O.A.C. and Mojonnier techniques.

Considerable attention has been given to the determination of lactose in milk. Babad & Grunpeter(460) deproteinize the milk with zinc sulphate and sodium hydroxide, and determine lactose in the filtrate by a modified Somogyi method. A colorimetric procedure described by Tulchinskiy(461) involves deproteination with aluminium sulphate, sodium hydroxide and sodium fluoride, and colour development of the filtrate heated with a potassium chromate-sodium carbonate mixture. Francis & Smith(462) deproteinize with colloidal iron, heat the filtrate with alkaline ferricyanide solution, and after cooling titrate with sulphato-ceric acid. Values are 2 to 3% higher than with Lane & Eynon's method and the A.O.A.C. gravimetric procedure. Oxidation with 0·5 N-$KMnO_4$ of the deproteinized filtrate is the basis of a method proposed by Indian workers(463). The cherry-red triphenylformazan which is produced when reducing sugars are heated with alkaline triphenyltetrazolium chloride may be applied to the determination of lactose in milk filtrates(464,465). Gorbach & Schäfer(466) clarify milk with zinc ferrocyanide and estimate lactose in the filtrate iodometrically. Estimation of the volume of precipitate in polarimetric determinations of lactose is described by Sarudi(467). The molybdenum blue method for

reducing sugars has been used by Potter(468) for studies of lactose hydrolysis in milk. In a study of the thermal decomposition of lactose in milk, Larsen & Gould(469) successfully used a photometric adaptation of the picric acid method for lactose. Reducing substances liberated from the heat-induced lactose-protein reaction were determined by Stewart(470) by the use of porphyroxide, a powerful oxidizing dye, in conjunction with *p*-aminoethylaniline sulphate. Larson & Jenness(471) have applied their method for protein sulphydryl groups to the determination of the reducing capacity of milk dialysates.

A rapid method for the determination of protein in milk, based on the direct distillation of milk with sodium hydroxide and barium chloride, is described by Slavyanov & Titov(472). Since neither fat nor lactose absorb iodine from aqueous solution, Indian workers(473) conclude that the absorption of iodine by milk is a reaction of the milk proteins. Milk diluted tenfold with distilled water is allowed to react for 6–7 hr. with excess 0·1 N-iodine. The excess iodine is determined by titration with thiosulphate, and the iodine absorption is equated to protein content by reference to a standard curve prepared from milks of known protein content. The formol titration values of milk and the corresponding whey increase as a result of lactic acid development by lactic streptococci, but are not altered by direct addition of lactic acid(474). Dumazert & Bozzi-Tichadou(475) determined casein in milk by precipitation with cadmium sulphate-sodium hydroxide, and obtained lower values by this method than by the customary acetic precipitation at the iso-electric point. Methods for the determination of total nitrogen and individual protein and non-protein constituents are given by Shahani & Sommer(476). The nitrogen distribution of milk as determined by the A.O.A.C., Rowland's and Harland & Ashworth's methods is the subject of an investigation by Ashworth & Drueger(477). While these three methods give comparable values for the non-protein and whey-protein fractions, they differ in the assessment of casein and proteose. The small amount of casein remaining in solution at the iso-electric point is included in the proteose fraction of the two former methods, but not in the latter.

Free tryptophane in unhydrolysed proteins is determined by Spies & Chambers(478) by condensation with *p*-dimethyl-amino-benzaldehyde followed by oxidation with sodium nitrite to give a blue coloration. With slight modifications, this method was used by Portner & Högl(479) to determine free and total tryptophane in foods. The percentage of total nitrogen as tryptophane was 1·5 in casein, and 1·3–1·8 in various types of cheese. In a further paper Spies & Chambers(480) determine amino-acids and peptides by conversion to their copper salts and transference of the copper to excess of alanine. The blue colour is measured spectrophotometrically at 620 m$\mu$. The method is suitable for studies in protein hydrolysis. Methods of determination of amino-acids are also given in Block & Bolling's publication(481). Various colour reactions of amino-acids are applied to dairy products by Thomasow as a means of detecting protein degradation(482).

The acidity test has been examined by Johnson & King(483), who have made several recommendations for improving its accuracy. From experience with incoming milk at a Transvaal city milk plant, Bakalor(484) concludes that much less milk would be rejected if a standard of pH 6·4 were adopted instead of 0·18% acidity, calculated as lactic acid. Musso(485) describes a test which has been in daily use for two years at a milk-receiving centre in Turin. Mixed with an equal volume of 0·2% haematoxylin in alcohol, normal milk gives a cherry-red colour corresponding to a pH of 6·5. As the acidity of the samples moves towards pH 5·6, so the colour changes through orange to orange-yellow.

A progress report on the chromatographic determination of lactic acid has been made by Mitchell(486). Lang & Pfleger(487) use a micro-method for lactic acid in which manganese dioxide replaces potassium permanganate as the oxidizing agent. In Paulsen's colorimetric method(488) the protein-free, lactose-free serum is treated with sulphuric acid, hydroxylamine hydrochloride and veratrole. Colour is developed at 25° C. for 20 min. The method is not sensitive to very small quantities of lactic acid. McAllister(489) describes a semi-quantitative colour reaction in which lactic acid is converted to acetaldehyde which is then coupled with azobenzene-phenylhydrazine sulphonic acid. The Barker-Summerson method for lactic acid has been applied successfully to ice cream and ice-cream ingredients by Neidlinger, Kempf & Stewart(490). Tiersma & Vene-

kamp(491) used the Heinemann method for the determination of lactic acid in milk and concluded that it was more suitable for research purposes than for routine laboratory analysis. For that reason Venekamp & Kruisheer(492) examined the possibilities of the well-known lactic acid-ferric chloride reaction when applied to a colloidal iron-milk filtrate. Working independently on the same line of approach Ling(493) had found this serum to give high 'blank' values, whereas a zinc sulphate-sodium hydroxide serum, free from sulphate ions through the inclusion of barium chloride in the precipitating mixture, gave the minimum blank colour when treated with ferric chloride. This forms the basis of Ling's method. As a rapid sorting test the milk serum may be treated with a mixed ferric ferroxyanide-ferric salicylate solution. The violet colour of this reagent changes to green and greenish yellow in the presence of small amounts of lactic acid.

Citric acid in milk is determined by Sarudi(494) with a slight modification of the pentabromoacetone procedure. Arakawa(495) has used the reaction between pyruvic acid and 2, 4-dinitro phenylhydrazine for the estimation of this acid in human milk. A method for the determination of individual ketone bodies in biological materials is described by Thin & Robertson(496). Two colorimetric methods for the determination of thiocyanates were compared by Wokes, Wedgwood & Wyatt(497). Analysis by both methods of over 100 samples showed that cow's milk contains 0·1–10 p.p.m. thiocyanate.

The determination of chloride by using diphenyl carbazone as indicator in the mercuric nitrate titration is considered by Borrell(498) to be more accurate than argentometric methods of titration. Buruiana & Niculesco(499) titrate the deproteinized milk filtrates of milk or cheese with mercuric nitrate using sodium nitroprusside as indicator. A rapid test for abnormally high chloride content of milk depends upon the addition of a mixture of silver nitrate, nitric acid and ferric alum of such concentration and balance that the addition of one drop of 0·1 N-potassium thiocyanate will give a red coloration in all samples containing more than 0·196% chloride(500). Pezzani(501) found 11·5–23 p.p.m. nitrate in human milk as determined by the nitro-xylenol method. Hänni(502) gives details of the quantitative determination of nitrate and nitrite in milk and cheese.

The flame photometer has been used for the determination of calcium, potassium and sodium in milk(503). Potassium may be estimated as the salt of tetraphenyl boron—$K(C_6H_5)_4B$(504). After destruction of organic matter by ashing or wet digestion, an aqueous solution of tetraphenyl boron is added followed by acetic acid. The precipitated potassium salt is dried at 120° C. Calcium ions were determined in milk ultracentrifugates by Smeets & Seekles(505) using their colour reaction with the ammonium salt of purpuric acid. On forty samples the mean value obtained was $110 \pm 16$ p.p.m. Magnesium in milk is estimated by Hunter(506) by adding to a suitable deproteinated filtrate more than sufficient of a thiazol yellow dye to form the magnesium complex. Colour measurement of the filtrate is used to determine the excess of the dye from which reference to a standard curve gives the magnesium concentration. Zinc determinations on milk kept in galvanized containers showed significant zinc contamination. A modified dithizone method was used(507). Streichówna(508) used a resorcinol method and observed that in the presence of air or iron, the rate of solution was much greater.

Brown(509) was able to resolve α-, β-, γ-, δ-tocopherols by filter-paper chromatography. Only α-tocopherol was found in the milk of cows on pasture. By means of a 30 cm. column of hydrated alumina Sternberg, Colas, & Kahn(510) were able to separate the major milk constituents.

The concentration of chloride and lactose in sour milk is considered by Müller(511) to be of more value in the detection of added water than specific gravity as determined on the re-liquified sample.

The degree of reflexion of light from the filter disk is used by Gerber & Siegenthaler(512, 513). for the determination of milk sediment. A photoelectric reflectometer is used for this purpose. The method is rapid and may be adapted to colour measurement of butter and dried milk. Scott(514) recognizes dung particles in the extraneous matter by staining with alcoholic safranine. The dung particles have a dull appearance from the mucilage adhering to their surfaces.

Quaternary ammonium compounds are estimated in milk or rinse waters by linking with

bromophenol blue with which they form insoluble blue compounds (515). Claborn & Beckman (516) have established a method for the determination of 0·25–1·0 p.p.m. methoxychlor in milk. Benzene hexachloride in milk may be estimated by conversion to trichlorobenzene which is conveniently measured by ultra-violet light absorption (517). The suggestion that the presence of antibiotics in milk may inactivate phosphatase and thus invalidate the customary test for efficient pasteurization (518) is not supported by the experiments of Churchill, Drury, Lewis, Frank & Bryan (519).

### (*b*) *Butter*

The common methods of routine analysis of butter are the subject of a revised specification of the British Standards Institution (520). Various methods for obtaining a homogeneous sample for analysis have been examined (521,522), and it is recommended that the sample should be softened at a temperature not greater than 39° C., and then mixed by hand shaking. Jax (523) has described a method for the rapid determination of moisture in butter, cheese and dried milk, in which the dishes are carried on a rotating vertical spindle enclosed in a cylindrical drying chamber. Some fundamental principles of entrainment distillation are presented by Dutch workers (524). The customary rapid determination of moisture in butter is made considerably more accurate and expeditious by the use of an oil-damped balance giving a direct reading of the weight of sample in excess of 9 g., and compensating for variations in sample weight (525). The relationship between the moisture content and the dielectric constant of butter has been used in a device fitted to the outlet of a continuous butter-making machine whereby the moisture content of the product was obtained with an accuracy of $\pm 0{\cdot}5\%$ (526). Moisture distribution in butter was determined microscopically by Müller (527) using butter smears 30 $\mu$ deep. Only droplets greater than 30 $\mu$ diameter were counted, as there was a definite correlation between their number and that of droplets ranging from 10 to 30 $\mu$ diameter. The droplet count was a reliable index of the amount of working of the butter, being less than 31,000 per ml. in well-worked butter and more than 170,000 per ml. in very under-worked butter. By a simple volumetric procedure the fat percentage of ten samples of butter was found to deviate 0–0·9% from gravimetric determinations on the same samples (528). The use of silica gel for the chromatographic determination of volatile fatty acids has been described by Nijkamp (529), and the paper chromatographic procedure has been reviewed by Gerritsma (530). The reliability of Hillig's method for the estimation of water-insoluble fatty acids has been the subject of four papers (531,532,533,534). Thirty-two commercial samples contained 126–612 mg. of water-insoluble acids per 100 g. fat, and although high values were generally associated with low butter scores, there were many inconsistencies (534). Armstrong & Harper (535) found that the α-naphtholphthalein method for measuring fat hydrolysis gave sufficiently close agreement with water-insoluble acid values to suggest that the method might provide a rapid means of assessing the degree of fat hydrolysis in butter or cream. Schaffer & Holm (536) describe the determination of linoleic acid in milk fat. In the iodometric estimation of peroxide values, the frequently encountered high blank values may be avoided by using a 10% solution of citric acid in tertiary butyl alcohol and carbon tetrachloride (537). Miller & Tracy (538) give a method for the estimation of copper in butter; samples made by the continuous process contained less copper than samples of batch-produced butter.

Three papers by Thaler & Scheler deal with the identification of colouring agents in butterfat. For the detection of annatto in butterfat and other edible fats an adsorption column of alumina, magnesium carbonate or similar basic adsorbent is used. The fixed annatto is not eluted by alcohol, petrol ether, benzene or chloroform and is thus easily separated from carotene, etc., and coal-tar dyes (539). A silicate column is used to remove butter-yellow from benzene solution (540). In the third paper the authors give details of the use of paper chromatography for the separation of annatto and twelve artificial colouring agents from edible fats (541). Diemair & Janecke (542) dissolve the unsaponifiable matter of butterfat in chloroform and use paper chromatography for the separation of annatto, carotene and butter-yellow.

### (*c*) *Cheese*

A revised publication of the British Standards Institution gives details of the commonly accepted methods of analysis of cheese (543). Recommendations for surmounting the difficulties of sampling soft cheese are made by Perlmutter & Horwitz (544). On the basis of 356 determinations of water and fat, Larsson (545) concludes that cheese may be sampled after pressing for 5–6 hr., since samples taken after 20 hr. gave no significant differences. Moisture in American processed cheese or cheese spread is determined by maceration in the moisture dish followed by vacuum drying at 100° C. for 5 hr. (546). Under these circumstances little caramelization or scorching is observed. A new approach to this particular problem is the use by Ozimov, Guygo & Alyamovskiy (547) of a wire gauze of 9 cm. diameter for taking a sample of the molten cheese, and the use of two diametrically opposed infra-red lamps of 250 W. each, to evaporate moisture. The gauze is suspended on a balance beam and weighed, and the lamps are brought into operation intermittently until no further loss of weight is observed. The procedure occupies only 3–4 min. and appears to be sufficiently accurate for routine purposes. Lodin & Buhrgard (548) determine moisture in wet curd by rolling it while still warm on filter-paper to remove surplus water, and by evaporating the remaining water on a hot plate, taking 2·7 g. of prepared curd for this purpose.

Various methods for the determination of fat in cheese have been compared by Schwarz, Mumm & Neumann (549). A new rapid method for fat in cheese (550) consists of treating the sample with α-chlor-naphthalene in which the fat dissolves. The resultant changes in specific gravity and refractive index of the oil are equated to fat percentages. In whey cheese, fat is determined by Buchholz & Smith (551) by solution in sulphuric acid sp. gr. 1·124 and extraction of the fat with a 1:4 ethyl ether:petroleum ether mixture. Lower fatty acids in cheese may be separated by silicic acid chromatography and estimated by titration of the fractions with standard alkali (552). Storgårds & Lindqvist (553) use filter-paper chromatography in a study of the free volatile acids in normal and defective cheese. The 'relative casein content' of cheese is used by Schulz & Mrowetz (554) to follow the course of ripening. This is the material precipitated from a solution of cheese in sodium pyrophosphate by saturated alum, and is composed of casein and its early breakdown products. In a rapid method for chlorides in cheese, Shakhbazyan (555) prepares a filtrate containing a few drops of concentrated nitric acid and some ferric thiocyanate. This is then titrated with N/20-mercurous nitrate.

### (*d*) *Dried milk, etc.*

A notable advance in the study of lactose crystallization in dried milk products has been made by Choi, Tatter & O'Malley (556,557), whose method for the determination of α-lactose hydrate is based on the fact that only the amorphous and β-lactose will dissolve when the powder is shaken with a saturated solution of lactose at 25° C. In spray- or roller-dried skim-milk powder with less than 4% moisture, the percentage of total lactose present as α-lactose hydrate ranged from 0·5 to 5·2, but higher moisture levels, 8–9%, caused a great increase to values varying between 39·6 and 63·2%. No increase in α-lactose hydrate was observed when dried whole milk was treated with 95% ethanol, and it is suggested that the known increase in extractable fat produced by this treatment is due to protein coagulation rather than lactose crystallization. Mohr & Koenen (558) point out that the addition of whey to skim milk before drying is best detected by the protein solids-not-fat ratio, which should be no less than 32·9/100. Genuine dry skim-milk powder contains up to 53·7% lactose. The alkalinity of the ash of dried milk continues to receive attention (559). Examination of the insoluble sediment in roller-dried skim-milk powder is greatly facilitated by prior digestion of the material with a pepsin-hydrochloric acid solution (560). The estimation of sucrose in sweetened condensed milk by Vuillaume (561) follows the sequence: protein precipitation, acid hydrolysis, oxidation of aldo-sugars by alkaline iodine, and cuprimetric determinations of fructose. Blanchard's procedure (562) is to determine lactose in a protein-free serum and to determine the total sugars after hydrolysis of another portion of the

serum. Bertrand's permanganate method for lactose is used in this work. The solubility of lactose and its hydrolytic products has been studied by Talley & Hunter(563). A mixture of all three in water at 25° C. gave the following solubilities: lactose 4·5%, glucose 39·6% and galactose 14·8%. Methods of analysis of condensed(564) and dried(565) milks are detailed in publications of the British Standards Institution.

The polarographic determination of zinc in dairy products by Khlopin, Litvinova & Privalova(566) follows wet ashing with nitric acid and hydrogen peroxide by extraction with 30% calcium chloride solution. For the estimation of lead in biological material, Blaxter & Allcroft(567) describe a rapid method, and report that the milk of a cow in late lactation contained 0·02 p.p.m. of lead. The milk of a ewe on the second day of lactation contained 0·11 p.p.m. A dose of 5 g. lead acetate 7 days before lambing increased the concentration of lead in the milk obtained on the second, fourth and sixth day post-partum to 0·28, 0·18 and 0·15 p.p.m. respectively(568). Using a colorimetric dithiozonate test, Meinsma(569) found that measurable quantities of lead passed into buttermilk from the plating of buckets and churns in the course of several hours.

Acidity development in cream was noticed by Devender, Lyon & Bird(570) to cause increases in the formol and ammonia titration values of both cream and the buttermilk derived therefrom. In dried buttermilk both these values were low and constant, and it was not, therefore, possible to use them as criteria of cream quality. The sampling and analysis of condensed buttermilk is the subject of a report by Bergman(571). Standards of composition of dairy products required in the U.S.A. are contained in a report of the Bureau of Dairy Industry(572). Horwitz(573) reports on slight modifications in procedure for routine methods of analysis of milk and milk products. Similar reports on standard methods of analysis used in the Netherlands(574,575) and in India(576,577) have appeared.

National Institute for Research in Dairying, University of Reading — R. Aschaffenburg

University of Nottingham School of Agriculture, Sutton Bonington — E. R. Ling

## REFERENCES

(1) Aschaffenburg & Rowland (1952). *J. Dairy Res.* **19**, 226.
(2) Moody, Wise, Parrish & Atkeson (1951). *J. Dairy Sci.* **34**, 106.
(3) Mantillaro, Ubertalle & Bosticco (1950). *Atti Soc. ital. Sci. vet.* **4**, 452.
(4) Bosticco, Ubertalle & Graziosi (1950). *Atti Soc. ital. Sci. vet.* **4**, 456.
(5) Bosticco, Ubertalle & Graziosi (1950). *Atti Soc. ital. Sci. vet.* **4**, 466.
(6) Bosticco, Graziosi & Ubertalle (1950). *Atti Soc. ital. Sci. vet.* **4**, 461.
(7) Rowland, Roy, Sears & Thompson (1953). *J. Dairy Res.* **20**, 16.
(8) Pope & Roy (1953). *Biochem. J.* **53**, 427.
(9) Provan (1950). *J. Brit. Dairy Fmrs' Ass.* **54**, 33.
(10) Rowland (1951). *J. R. sanit. Inst.* **71**, 255.
(11) Bartlett & Kay (1950). *J. R. agric. Soc.* **111**, 87.
(12) Davis (1952). *Analyst*, **77**, 499.
(13) Balch, Balch, Bartlett, Cox & Rowland (1952). *J. Dairy Res.* **19**, 39.
(14) Bailey (1952). *J. Dairy Res.* **19**, 89, 102, 109, 115.
(15) Bailey (1952). *J. Dairy Res.* **19**, 169.
(16) Mahadevan (1951). *J. agric. Sci.* **41**, 80.
(17) Edwards (1951). *J. agric. Sci.* **40**, 100.
(18) Featherstone, Rickaby & Cavell (1951). *J. Dairy Res.* **18**, 155.
(19) McLean (1951). *Tech. Publ. Lincoln Coll., Canterbury agric. Coll., Univ. N.Z.* **7**.
(20) Kublitz (1952). *Milchwissenschaft*, **7**, 197.
(21) Nichols & Few (1950). *Qd. J. agric. Sci.* **7**, 49.
(22) Kielbasińska (1950). *Roczn. Zakl. Hig., Warsz.*, **1**, 173.
(23) Antoine (1952). *Bull. Inst. agron. Gembloux*, **20**, 5.

(24) Lonka (1951). *Maataloust. Aikakausk.* **24**, 176.
(25) Stüber & Göbelt (1952). *Milchwiss. Berichte, Wolfpassing*, no. **1**, 1.
(26) Dicks, Eaton & Carpenter (1951). *J. Dairy Sci.* **34**, 52.
(27) Johansson, Korkman & Nelson (1952). *Acta agric. Scand.* **2**, 43.
(28) Johansson (1952). *Acta agric. Scand.* **2**, 82.
(29) Korkman (1950). *Z. Tierz. ZüchtBiol.* **59**, 98.
(30) Vereshchagina (1950). *Sovetsk. Zootekhn.* **5**, (8), 8.
(31) Patow (1950). *Züchtungskunde*, **21**, 218.
(32) Malyoth, Stein, Hörmann & Schuler (1953). *Experientia*, **9**, 70.
(33) Krukovsky & Loosli (1952). *J. Dairy Sci.* **35**, 834.
(34) Ormsbee (1951). *Publ. Hlth Rep., Wash.*, **66**, 1685.
(35) Schmid & Zollikofer (1952). *Schweiz. Milchztg*, **78**, 129.
(36) Aas (1947). *Proc. int. Congr. pure appl. Chem., London*, **3**, 1.
(37) Gulvady, Kannan & Basu (1952). *Indian J. Dairy Sci.* **5**, 125.
(38) Khambatta & Dastur (1951). *Indian J. Dairy Sci.* **4**, 73.
(39) Venkatappaiah & Basu (1952). *Indian J. Dairy Sci.* **5**, 95.
(40) Bakalor (1948). *Bull. Dep. Agric. S. Afr.* **285**.
(41) Bakalor (1948). *Bull. Dep. Agric. S. Afr.* **301**.
(42) Bakalor (1951). *The Compositional Quality of the South African Milk Supply.* Mimeograph, Agric. Res. Inst., Pretoria, South Africa.
(43) Parisi (1949). *Riv. Zootec., Firenze*, **22**, 159.
(44) Fabris (1951). *Mondo Latte*, p. 460.
(45) Carbone (1952). *Boll. Agric., Milano*, **1952**, no. 26.
(46) Shvabe (1951). *Proc. Lenin Acad. agric. Sci.* **16**, 39.
(47) Jack, Roessler, Abbott & Irwin (1951). *Bull. Calif. agric. Exp. Sta.* no. 726.
(48) Uesaka, Yahata & Imamura (1951). *Bull. Res. Inst. Food Sci., Kyoto Univ.*, **6**, 7.
(49) Uesaka & Tze-Yee Chang (1951). *Bull. Res. Inst. Food Sci., Kyoto Univ.*, **6**, 13.
(50) Ellenberger, Newlander & Jones (1950). *Bull. Vt agric. Exp. Sta.* no. 556.
(51) Munch-Petersen (1950). *Nord. VetMed.* **2**, 697.
(52) Rowallan, Lord (1950). *Fmr & Stk-Breed.* **64**, 2679, 2707; (1951), **65**, 669.
(53) Bartlett, Cowie, Rowland & Allcroft (1951). *Fmr & Stk-Breed.* **65**, 259.
(54) Bartlett, Palmer, Rowland & Allcroft (1951). *Fmr & Stk-Breed.* **65**, 41.
(55) Archibald (1951). *J. Dairy Sci.* **34**, 1026.
(56) Casini (1946). *Ann. Chim. appl., Roma*, **36**, 219.
(57) Lengemann, Aines & Smith (1952). *Cornell Vet.* **42**, 28.
(58) Fabris (1951). *Mondo Latte*, **1951**, 598.
(59) Asdrubali (1950). *Zooprofilassi*, **5**, 385.
(60) Breirem, Ender, Halse & Slagsvold (1949). *Acta agric. suec.* **3**, 89.
(61) Valen & Valen (1950). *Meieriposten*, **39**, 793.
(62) Ulvesli, Husby & Presthegge (1952). *Tidsskr. norske Landbr.* **59**, 118.
(63) Neseni & Freimuth (1952). *Milchwissenschaft*, **7**, 113.
(64) Seidler (1951). *Milchwissenschaft*, **6**, 236.
(65) Seidler (1951). *Milchwissenschaft*, **6**, 58.
(66) Principalle (1948). *Ann. Chim. appl., Roma*, **38**, 617.
(67) Neseni & Freimuth (1951). *Züchtungskunde*, **23**, 133.
(68) Heidebrecht, Macvicar, Ross & Whitehair (1951). *J. Nutr.* **44**, 43.
(69) Sheffy, Shahani, Grummer, Phillips & Sommer (1952). *J. Nutr.* **48**, 103.
(70) Smith (1952). *N.Z. J. Sci. Tech.* **34**, sect. A, p. 65.
(71) Beacom & Bowland (1951). *J. Nutr.* **45**, 419.
(72) El-Sokkary, Sirry & Hassan (1949). *J. agric. Sci.* **39**, 287.
(73) Adam (1950). *Zir. Fak. Yayinl.* **9**.
(74) Thomsen (1951). *Butt. Cheese Milk Prod. J.* **42**, (8), 28.
(75) Tuomainen (1947). *Meijeriteit. Aikakausk.* **9**, 70.
(76) Tammisto & Tuomainen (1948). *Karjantuotteesta*, no. 19.
(77) Evenhuis (1952). *Neth. Milk Dairy J.* **6**, 137.

(78) Hillig, Ahlmann & Jordan (1951). *J. Ass. off. agric. Chem. Wash.* **34**, 720.
(79) Morgan, Anderson, Hankin, Dowd & Foter (1952). *Bull. Storrs agric. Exp. Sta.* no. 284.
(80) Schulz, Thomasow & Leder (1952). *Kieler Milchwirtschaft. ForschBerichte*, **4**, 341.
(81) Irvine (1951). *Canad. Dairy Ice Cr. J.* **30**, (7), 27.
(82) McDowall (1952). *N.Z. J. Sci. Tech.* **33**, sect. A, 29.
(83) Fagen, Stine & Hussong (1952). *J. Dairy Sci.* **35**, 779.
(84) Trautman, Hansen & Hartwell (1951). *Proc. W. Div. Amer. Dairy Sci. Ass.* p. 74.
(85) Kosikowsky (1951). *J. Dairy Sci.* **34**, 228.
(86) Kosikowsky (1951). *J. Dairy Sci.* **34**, 235.
(87) Fagen & Hussong (1952). *J. Dairy Sci.* **35**, 479.
(88) Uuspää & Torsti (1951). *Ann. Med. exp. Biol. fenn.* **29**, 58.
(89) Hiscox, Harrison & Wolf (1951). *J. Dairy Res.* **18**, 296.
(90) Krett, Leboyer, Daume & Stine (1952). *J. Dairy Sci.* **35**, 480.
(91) Mattick (1951). *J. Dairy Res.* **18**, 305.
(92) Orlova (1950). *Probl. Pediat., Leningr.*, **18**, 50.
(93) Skilladziowa, Wojna, Rudowska & Łuczakowa (1950). *Roczn. Zakl. Hig., Warsz.*, **1**, 155.
(94) Randoin & Jourdan-Vatinel (1951). *Lait*, **31**, 113, 150, 617.
(95) Randoin & Jourdan-Vatinel (1952). *Lait*, **32**, 481, 593, 607.
(96) Harper & Gould (1952). *Butt. Cheese Milk Prod. J.* **43**, (5), 24.
(97) Harper (1952). *J. Dairy Sci.* **35**, 480.
(98) Schwarz & Mumm (1949). *Milchwissenschaft*, **4**, 382.
(99) Schwarz & Thomasow (1950). *Milchwissenschaft*, **5**, 376.
(100) Schwarz, Castillo & Mumm (1952). *Kieler Milchwirtschaft. ForschBerichte*, **4**, 243.
(101) Maxa (1949). *Výz. lidu*, **4**, 40.
(102) Rudowska, Szkilladziowa, Łuczakowa, Berger & Wojna (1951). *Roczn. Zakl. Hig., Warsz.*, **2**, 69.
(103) Patton (1952). *J. Dairy Sci.* **35**, 324.
(104) Greenbank (1952). *J. Dairy Sci.* **35**, 486.
(105) McClymont (1951). *Aust. J. agric. Res.* **2**, 158.
(106) Murthy, Lalitha & Dastur (1951). *Indian J. Dairy Sci.* **4**, 129.
(107) Achaya & Hilditch (1950). *Proc. roy. Soc.* B, **137**, 187.
(108) Bartley, Zaletel, Bird, Cannon, Wise & Kempthorne (1951). *J. Dairy Sci.* **34**, 536.
(109) Hilditch (1950). *Brit. J. Nutr.* **3**, 347.
(110) Shorland (1950). *Nature, Lond.*, **166**, 745.
(111) Shorland & Johannesson (1951). *Nature, Lond.*, **168**, 75.
(112) Hansen, Shorland & Cooke (1951). *Chem. & Ind., Lond.*, no. 40, p. 839.
(113) Hansen & Shorland (1951). *Biochem. J.* **50**, 207, 358.
(114) Hansen & Shorland (1952). *Biochem. J.* **52**, 207.
(115) McDowell (1953). *J. Dairy Res.* **20**, 101.
(116) Hove & Harris (1951). *J. Amer. Oil Chem. Soc.* **28**, 405.
(117) Cerutti (1953). *Ann. Sper. agr.* **7**, 189.
(118) Mattsson, Thomé & Swartling (1952). *Medd. Mejeriförs.* no. 35.
(119) Bergman & Joost (1952). *Mejeritek. Medd.* **13**, 23.
(120) Morice (1951). *J. chem. Soc.* p. 1200.
(121) Sheffy, Phillips, Dymsza, Grummer & Bohstedt (1952). *J. Anim. Sci.* **11**, 727.
(122) Kofranyi (1951). *Hoppe-Seyl. Z.* **287**, 170.
(123) Shaháni & Sommer (1951). *J. Dairy Sci.* **34**, 1010, 1035.
(124) Springer & Quentin (1951). *Biochem. Z.* **322**, 180.
(125) Block (1951). *J. Dairy Sci.* **34**, 1.
(126) Hodson (1950). *Industr. Engng Chem.* **42**, 694.
(127) Schormüller (1951). *Dtsch. Lebensmitt. Rdsch.* **47**, 175.
(128) Lea, Rhodes & Borrell (1952). *Nature, Lond.*, **169**, 1097.
(129) Polonovski & Robert (1951). *Bull. Soc. Chim. biol., Paris*, **33**, 1199.
(130) Polonovski & Robert (1951). *Bull. Soc. Chim. biol., Paris*, **33**, 1174.
(131) Barnabei & Mari (1950). *Quad. Nutriz.* **11**, 17.
132) Morton (1950). *Nature, Lond.*, **166**, 1092.

(133) Zittle & DellaMonica (1952). *Arch. Biochem. Biophys.* **35**, 321.
(134) Zittle & DellaMonica (1951). *Proc. Soc. exp. Biol., N.Y.*, **76**, 193.
(135) Zittle & DellaMonica (1950). *Arch. Biochem.* **26**, 112.
(136) Zittle & DellaMonica (1950). *Arch. Biochem.* **26**, 135.
(137) Chanda & Owen (1952). *J. agric. Sci.* **42**, 403.
(138) Chanda, Owen & Cramond (1951). *Brit. J. Nutr.* **5**, 228.
(139) Chanda, Owen & Watts (1951). *Biochem. J.* **50**, ii.
(140) Chanda & Owen (1951). *Biochem. J.* **50**, 100.
(141) Kannan & Basu (1951). *Indian J. Dairy Sci.* **4**, 8.
(142) Hansen, Wood & Thornton (1952). *J. Dairy Sci.* **35**, 487.
(143) Lear & Foster (1951). *J. Milk Food Tech.* **14**, 131.
(144) Cook & Hughes (1952). *Mon. Bull. Minist. Hlth Lab. Serv.* **11**, 254.
(145) Wight & Tramer (1952). *Dairy Ind.* **17**, 54.
(146) Aschaffenburg (1953). *Dairy Ind.* **18**, 316.
(147) Bessey & Love (1952). *J. biol. Chem.* **196**, 175.
(148) Aschaffenburg (1953). *Science*, **117**, 611.
(149) Burgwald (1950). *Rep. N.Y. St. Ass. Milk Sanit.* p. 29.
(150) Schwarz (1952). *Z. LebensmittUntersuch.* **94**, 88.
(151) Schwarz & Lange (1951). *Süddtsch. Molkereiztg*, **72**, 479.
(152) Kieferle & Seuss (1951). *Molkereiztg, Hildesh.*, **5**, 412.
(153) Schwarz & Lange (1951). *Dtsch. Molkereitzg*, **72**, 1094, 1124.
(154) Wagener & Erberhardt (1951). *Milchwissenschaft*, **6**, 355.
(155) Beck (1951). *Milchwissenschaft*, **6**, 387.
(156) Seelemann (1951). *Grosstädt. Milchversorg.* **1951**, no. 10, 67.
(157) Jarrousse, Lambert & Petransxiène (1950). *C.R. Acad. Agric. Fr.* **36**, 548.
(158) Jarrousse, Lambert & Petransxiène (1950). *C.R. Acad. Agric. Fr.* **36**, 551.
(159) Posthumus (1952). *Off. Org. K. ned. Zuivelb.* **44**, 458.
(160) Simskaya (1952). *Gigiena i Sanit.* no. 2, p. 33.
(161) Simskaya (1952). *Gigiena i Sanit.* no. 5, p. 34.
(162) Camus & Alifax (1951). *Lait*, **31**, 367.
(163) Håkansson & Sjöström (1952). *Svenska Mejeritidn.* **44**, (2), 15.
(164) Jacquet & Saingt (1952). *C.R. Soc. Biol., Paris*, **146**, 1515.
(165) Schwander, Zahler & Nitschmann (1952). *Helv. chim. acta*, **35**, 553.
(166) Nitschmann & Varin (1951). *Helv. chim. acta*, **34**, 1421.
(167) Mattenheimer, Nitschmann & Zahler (1952). *Helv. chim. acta*, **35**, 1970.
(168) Nitschmann & Zahler (1950). *Helv. chim. acta*, **33**, 854.
(169) Hostettler & Rüegger (1950). *Annu. agric. Suisse*, **51**, 669.
(170) Hostettler & Imhof (1952). *Annu. agric. Suisse* (new series), **1**, 307.
(171) Varin (1951). Thesis, Bern.
(172) Baud, Morard & Pernoux (1951). *C.R. Acad. Sci., Paris*, **233**, 276.
(173) McKerns (1951). *Canad. J. med. Sci.* **29**, 59.
(174) D'yachenko (1950). *Mol. Prom.* **11**, (6), 27.
(175) Schulz (1951). *Milchwissenschaft*, **6**, 74, 112.
(176) Zimmerman (1951). *Iowa St. Coll. J. Sci.* **25**, 389.
(177) Berridge (1952). *Analyst*, **77**, 57.
(178) Berridge (1952). *J. Dairy Res.* **19**, 328.
(179) Titov (1949). *Myasn. Ind. S.S.S.R.* **20**, (4), 38.
(180) Salati (1952). *Mondo d. Latte*, p. 482.
(181) Pereira de Matos & Vieira de Sá (1948). *Bol. pecvar.* **16**, (1).
(182) Krishnamurti & Subrahmanyan (1949). *Indian J. Dairy Sci.* **2**, 19.
(183) Gonashvili (1952). *Mol. Prom.* **13**, (1), 37.
(184) Herrington (1950). *Conv. Proc. Milk Ind. Found.* **1950**, Vol. **4**, 30.
(185) Kannan & Basu (1951). *Indian J. Dairy Sci.* **4**, 63.
(186) Lubert, Smith & Thornton (1949). *Canad. J. Res.* **27**, F, 483.
(187) Lubert, Smith & Thornton (1949). *Canad. J. Res.* **27**, F, 499.

(188) Loncin & Jacqmain (1951). *Lait*, **31**, 233.
(189) Polis & Shmukler (1953). *J. biol. Chem.* **201**, 475.
(190) Chance (1950). *J. Amer. chem. Soc.* **72**, 1577.
(191) Burbelo (1950). Thesis, Hannover.
(192) Van der Smissen (1951). *Kieler Milchwirtschaft. ForschBerichte*, **3**, 73.
(193) Schulz & Sydow (1951). *Kieler Milchwirtschaft. ForschBerichte*, **3**, 673.
(194) Buruiana (1950). *Lait.* **30**, 2.
(195) Buruiana & Geraniou (1951). *Lait*, **31**, 134.
(196) Hofstee (1949). *J. biol. Chem.* **179**, 633.
(197) Kalckar, Kjeldgaard & Klenow (1950). *Biochim. biophys. Acta*, **5**, 575.
(198) Morell (1952). *Biochem. J.* **51**, 657.
(199) Harris & Hellerman (1953). *Fed. Proc.* (Amer. Soc. Exp. Biol.) **12**, 215.
(200) Polonovski, Baudu, Robert & Robert (1950). *Bull. Soc. Chim. biol., Paris*, **32**, 855.
(201) Polonovski, Robert & Robert (1950). *Bull. Soc. Chim. biol., Paris*, **32**, 862.
(202) Polonovski, Robert & Robert (1950). *Bull. Soc. Chim. biol., Paris*, **32**, 868.
(203) Polis & Shmukler (1950). *J. Dairy Sci.* **33**, 619.
(204) Lemacher, Merten & Ratzer (1950). *Arch. Gynäk.* **179**, 94.
(205) Laskowski & Laskowski (1951). *J. biol. Chem.* **190**, 563.
(206) Laskowski, Mars & Laskowski (1952). *J. biol. Chem.* **198**, 745.
(207) Lembke & Christiansen (1951). *Kieler Milchwirtschaft. ForschBerichte*, **3**, 385.
(208) Hung-Chun Kung, Gaden & King (1952). *Abstr. Pap. Amer. chem. Soc.* Sept. no. 21 A.
(209) Kannan & Basu (1951). *Indian J. Dairy Sci.* **4**, 115.
(210) Paschke (1952). *Milchwissenschaft*, **7**, 3.
(211) Tobias, Whitney & Tracy (1952). *J. Dairy Sci.* **35**, 1026.
(212) Tobias, Whitney & Tracy (1952). *J. Dairy Sci.* **35**, 1036.
(213) Slatter & Van Winkle (1952). *J. Dairy Sci.* **35**, 1083.
(214) Heyndrickx & de Vleeschauwer (1952). *Experientia*, **8**, 317.
(215) Heyndrickx & de Vleeschauwer (1951). *Biochim. biophys. Acta*, **6**, 487.
(216) Heyndrickx & de Vleeschauwer (1952). *Bull. Soc. Chim. biol., Paris*, **34**, 1016.
(217) Barry (1952). *J. biol. Chem.* **195**, 795.
(218) Work, Campbell & Askonas (1952). *Congr. int. Biochim.* p. 50.
(219) Campbell & Work (1952). *Biochem. J.* **52**, 217.
(220) Hare, Schwartz & Weese (1952). *J. Dairy Sci.* **35**, 615.
(221) Nitschmann (1949). *Helv. chim. acta*, **32**, 1258.
(222) Vlodavets, D'yachenko & Bogomolova (1952). *Mol. Prom.* **13**, (7), 33.
(223) D'yachenko & Vlodavets (1952). *Colloid J., Voronezh.*, **14**, 338.
(224) McMeekin, Groves & Hipp (1949). *J. Amer. chem. Soc.* **71**, 3298.
(225) McMeekin & Marshall (1952). *Science*, **116**, 142.
(226) Hipp, Groves, Custer & McMeekin (1950). *J. Amer. chem. Soc.* **72**, 4928.
(227) Hipp, Groves, Custer & McMeekin (1952). *J. Dairy Sci.* **35**, 272.
(228) Zweig & Block (1953). *Fed. Proc.* (Amer. Soc. Exp. Biol.) **12**, 296.
(229) Cherbuliez & Baudet (1950). *Helv. chim. acta*, **33**, 398.
(230) Cherbuliez & Baudet (1950). *Helv. chim. acta*, **33**, 1673.
(231) Nitschmann & Zürcher (1950). *Helv. chim. acta*, **33**, 1698.
(232) Dovey & Campbell (1952). *Nature, Lond.*, **169**, 1014.
(233) Kamal & Turner (1951). *J. Dairy Sci.* **34**, 790.
(234) Ter Horst (1950). *Neth. Milk Dairy J.* **4**, 246.
(235) Higgins & Hayes (1951). *Aust. J. appl. Sci.* **2**, 368.
(236) Brintzinger & Wolff (1950). *Kolloidzschr.* **118**, 26.
(237) Kiermeier (1952). *Z. LebensmittUntersuch.* **95**, 85.
(238) Ovchinnikov (1951). *Mol. Prom.* **12**, (6), 28.
(239) Lust (1952). *Lait*, **32**, 241.
(240) Plomley, Higgins & Hayes (1951). *Nature, Lond.*, **167**, 224.
(241) Higgins, Fraser & Hayes (1952). *Nature, Lond.*, **169**, 1020.
(242) Wormell (1951). *Nature, Lond.*, **167**, 817.

(243) Mellon, Korn, Kokes & Hoover (1951). *J. Amer. chem. Soc.* **73**, 1870.
(244) Hoover (1951). *Abstr. Pap. Amer. chem. Soc.* April, no. 25C.
(245) Mellon, Korn & Hoover (1952). *Abstr. Pap. Amer. chem. Soc.* Sept. no. 48C.
(246) Fraser & Powell (1950). *J. biol. Chem.* **187**, 803.
(247) Perlmann (1952). *J. Amer. chem. Soc.* **74**, 3191.
(248) Pedersen & Baker (1952). *Nature, Lond.*, **169**, 928.
(249) Paulson, Deatherage & Almy (1952). *Abstr. Pap. Amer. chem. Soc.* Sept. no. 49C.
(250) Karambelkar, Ramasarma & Sreenivasan (1950). *Ann. Biochem. exp. Med.* **10**, 1.
(251) Pedersen (1949). *Beretn. Forsøgsm., Kbh.*, no. **56**.
(252) Kastens & Baldauski (1952). *Industr. Engng Chem.* **44**, 1257.
(253) Simonart & Mayaudon (1952). *Neth. Milk Dairy J.* **6**, 1.
(254) Storgårds & Lindqvist (1953). *Milchwissenschaft*, **8**, 5.
(255) Storgårds (1953). *Svenska Mejeritidn.* **45**, (4), 37; (5), 51.
(256) Wegelin (1952). *Versl. landb. Onderzoek., Minist. Landb., 's Grav.*, **58.3**.
(257) Schäfer (1951). *Mschr. Kinderheilk.* **99**, 69.
(258) Stanley, Whitnah & Andrews (1951). *J. Dairy Sci.* **34**, 265.
(259) Hellbrügge (1951). *Mschr. Kinderheilk.* **99**, 76.
(260) Polis, Shmukler, & Custer (1950). *J. biol. Chem.* **187**, 349.
(261) Coulson & Stevens (1950). *J. biol. Chem.* **187**, 355.
(262) Gordon & Semmett (1953). *J. Amer. chem. Soc.* **75**, 328.
(263) Weinstein, Duncan & Trout (1951). *J. Dairy Sci.* **34**, 570.
(264) Weinstein, Lillevik, Duncan & Trout (1951). *J. Dairy Sci.* **34**, 784.
(265) Harland, Coulter & Jenness (1952). *J. Dairy Sci.* **35**, 363.
(266) Hutton & Patton (1952). *J. Dairy Sci.* **35**, 699.
(267) Johnson & Swanson (1952). *J. Dairy Sci.* **35**, 823.
(268) Heinemann & Parker (1951). *J. Dairy Sci.* **34**, 427.
(269) Dawson (1951). *Nature, Lond.*, **168**, 241.
(270) Riley (1951). *Nature, Lond.*, **168**, 242.
(271) Halwer, Nutting & Brice (1951). *J. Amer. chem. Soc.* **73**, 2786.
(272) Ritland, Kaesberg & Beeman (1950). *J. chem. Phys.* **18**, 1237.
(273) Jacobsen (1949). *C.R. Lab. Carlsberg* (Sér. chim.), **26**, 455.
(274) Polis, Schmukler, Custer & McMeekin (1950). *J. Amer. chem. Soc.* **72**, 4965.
(275) Groves, Hipp & McMeekin (1952). *Fed. Proc.* (Amer. Soc. Exp. Biol.), **11** (1, Part I), 223.
(276) McMeekin, Polis, DellaMonica & Custer (1949). *J. Amer. chem. Soc.* **71**, 3606.
(277) Groves, Hipp & McMeekin (1951). *J. Amer. chem. Soc.* **73**, 2790.
(278) Weil & Buchert (1951). *Arch. Biochem. Biophys.* **34**, 1.
(279) Larson & Jenness (1952). *J. Amer. chem. Soc.* **74**, 3090.
(280) McFarren & Mills (1952). *Analyt. Chem.* **24**, 650.
(281) McDougall (1949). *Biochem. J.* **44**, 531.
(282) Smith & Brown (1950). *J. biol. Chem.* **183**, 241.
(283) Aschaffenburg, Bartlett, Kon, Roy, Walker, Briggs & Lovell (1951). *Brit. J. Nutr.* **5**, 171.
(284) Foster, Friedell, Catron & Dieckmann (1951). *Arch. Biochem. Biophys.* **31**, 104.
(285) McIntyre, Parrish & Fountaine (1952). *J. Dairy Sci.* **35**, 356.
(286) Schmid (1951). *Milchwissenschaft.* **6**, 424.
(287) Odenwald (1952). *Züchtungskunde*, **23**, 151.
(288) Fialkov (1952). *Mol. Prom.* **13**, (7), 36.
(289) Rangappa & Banerjee (1950). *Indian J. vet. Sci.* **20**, 123.
(290) Whittlestone (1952). *J. Dairy Res.* **19**, 127.
(291) Whittlestone (1952). *J. Dairy Res.* **19**, 335.
(292) Piskarev & Kremnev (1951). *Mol. Prom.* **12**, (10), 31.
(293) Ashworth (1951). *J. Dairy Sci.* **34**, 317.
(294) King (1951). *Dairy Ind.* **16**, 727.
(295) King (1950). *Svenska Mejeritidn.* **42**, 507.
(296) King (1951). *Medd. Mejeriförsök*, no. 30.
(297) Mohr & Kaufmann (1950). *Fette u. Seifen*, **52**, 537.

(298) Rishoi (1952). *J. Dairy Sci.* **35**, 1125.
(299) Nickerson, Coulter & Jenness (1952). *J. Dairy Sci.* **35**, 77.
(300) Davidov (1952). *Mol. Prom.* **13**, (1), 29.
(301) Antoniani, Cerutti & Vedani (1951). *Ann. sper. agr.* **5**, 1405.
(302) Antoniani & Cerutti (1952). *Ann. Sper. agr.* **6**, 651.
(303) Farfaletti-Casali & Cerutti (1953). *Ann. Sper. agr.* **7**, 193.
(304) Cerutti (1952). *Ann. Sper. agr.* **7**, 1493.
(305) Babcock, Strobel, Yager & Windham (1952). *J. Dairy Sci.* **35**, 195.
(306) Haller & Bell (1950). *Milk Dlr*, **40**, (1), 98.
(307) Bell & Mucha (1951). *J. Dairy Sci.* **34**, 432.
(308) Mucha & Bell (1951). *J. Dairy Sci.* **34**, 953.
(309) Doan (1952). *Food Tech., Champaign, Ill.*, **6**, 402.
(310) Wildasin & Doan (1951). *J. Dairy Sci.* **34**, 438.
(311) Bell & Mucha (1952). *J. Dairy Sci.* **35**, 1.
(312) Grant (1952). [Mimeograph] U.S. Dep. Agric. BDI-Inf-136.
(313) Davidov & Barabanshchikov (1951). *Mol. Prom.* **12**, (1), 28.
(314) Refrigeration Research Foundation (1952). [Mimeograph] Colorado.
(315) Ramakrishnan & Banerjee (1951). *Research*, **4**, 241.
(316) Ramakrishnan & Banerjee (1952). *Indian J. Dairy Sci.* **5**, 25.
(317) Cacioppo (1950). *Pediatria, Napoli*, **58**, 371.
(318) Roeder (1950). *Milchwissenschaft*, **5**, 391, 426.
(319) Roeder (1951). *Milchwissenschaft*, **6**, 54, 91, 165.
(320) Hansson (1951). *Mejeritek. Medd.* **1951**, (5), 89.
(321) Krauss (1950). *Kieler Milchwirtschaft. ForschBerichte*, **2**, 127.
(322) Kermack & Miller (1951). *Arch. Dis. Childh.* **26**, 265, 320.
(323) Miller (1951). *Arch. Dis. Childh.* **26**, 325.
(324) Miller & Jackson (1951). *Arch. Dis. Childh.* **26**, 329.
(325) Mohr & Corte (1950). *Kieler Milchwirtschaft. ForschBerichte*, **2**, 13.
(326) Mohr & Corte (1950). *Kieler Milchwirtschaft. ForschBerichte*, **2**, 153.
(327) Schaffer (1949). *Ann. Hyg. publ., Paris*, **27**, 207.
(328) Riedel (1949). *Chem.-Ing.-Tech.* **21**, 340.
(329) Patton & Josephson (1952). *J. Dairy Sci.* **35**, 161.
(330) Strogyll, Gould & Johnson (1951). *J. Dairy Sci.* **34**, 219.
(331) Patton (1950). *J. Dairy Sci.* **33**, 904.
(332) Patton (1952). *J. Dairy Sci.* **35**, 1053.
(333) Tarassuk & Simonson (1950). *Food Tech., Champaign, Ill.*, **4**, 88.
(334) Moster & Chapman (1949). *Canad. J. Res.* **27**, Sect. F, 429.
(335) Lea & Hannan (1949). *Biochim. biophys. Acta*, **3**, 313.
(336) Lea & Hannan (1950). *Biochim. biophys. Acta*, **4**, 518.
(337) Lea & Hannan (1950). *Biochim. biophys. Acta*, **5**, 433.
(338) Lea, Hannan & Rhodes (1951). *Biochim. biophys. Acta*, **7**, 366.
(339) Lea & Rhodes (1952). *Biochim. biophys. Acta*, **9**, 56.
(340) Hannan & Lea (1952). *Biochim. biophys. Acta*, **9**, 293.
(341) Lewis & Lea (1950). *Biochim. biophys. Acta*, **4**, 532.
(342) Lea (1950). *Chem. & Ind., Lond.*, no. 9, p. 155.
(343) Brunner (1950). *J. Dairy Sci.* **33**, 741.
(344) Panasenkov (1952). *Mol. Prom.* **13**, (2), 26.
(345) Janzen & Swanson (1952). *J. Dairy Sci.* **35**, 482.
(346) Hollender (1952). *J. Dairy Sci.* **35**, 482.
(347) Griebel & Schwarz (1952). *Dtsch. Lebensmitt-Rdsch.* **48**, (5), 95.
(348) Deysher & Webb (1952). *J. Dairy Sci.* **35**, 106.
(349) Lawrence (1952). *Aust. J. Dairy Tech.* **7**, 52.
(350) Bergum (1950). *Beretn. Sta. Meieriforsøk (Norg. LandbrHøgsk.)*, no. 41.
(351) Alekseeva & Kasatochkin (1951). *Izv. Akad. Nauk. S.S.S.R., Otdel. Fiz.*, **15**, 222.
(352) McGlasson & Boyd (1951). *J. Dairy Sci.* **34**, 119.

(353) Arrington & Krienke (1949). *Proc. Sth agric. Wkrs*, p. 77. (408)
(354) Dharamarajan, Venkateswara Rao, Menon & Dastur (1950). *Indian J. vet. Sci.* **20**, 35. (409)
(355) Venkateswara Rao, Dastur & Dharamarajan (1950). *Indian J. vet. Sci.* **20**, 263. (410)
(356) Rees (1952). *Res. Ser. Bull. Tasm. Dep. Agric.* **1**. (411)
(357) Lythgoe (1952). *J. Ass. off. agric. Chem., Wash.*, **35**, 442. (412)
(358) Lythgoe (1952). *J. Ass. off. agric. Chem., Wash.*, **35**, 855. (413)
(359) Paley & Tzall (1950). *Rep. N.Y. St. Ass. Milk Sanit.* p. 81. (414)
(360) Rothman (1951). *Rep. N.Y. St. Ass. Milk Sanit.* p. 93. (415)
(361) Ystgaard, Homeyer & Bird (1951). *J. Dairy Sci.* **34**, 680, 689. (416)
(362) Hoffmann (1951). *J. Ass. off. agric. Chem., Wash.*, **34**, 248. (417)
(363) Sager (1951). *Z. Kinderheilk.* **70**, 319. (418)
(364) Mahajan & Mathur (1949). *Curr. Sci.* **18**, 204. (419)
(365) Goded y Mur (1951). *An. Bromatol.* **3**, 267. (420)
(366) Bena-Medean & Liteanu (1948). *Bull. Soc. Sti. Cluj*, **10**, 100. (421)
(367) Stoldt (1949). *Z. LebensmittUntersuch.* **89**, 47. (422)
(368) Alison (1952). *Lait*, **32**, 258. (423)
(369) Schwarz & Effern (1950). *Molkereiztg Hildesh.* **4**, 899. (424)
(370) Hadland & Solberg (1952). *Nord. JordbrForskn.* **34**, 36. (425)
(371) Isidoro & Pavolini (1950). *Boll. Lab. chim. provinciali (Bologna)*, **1**, (4), 18. (426)
(372) Isidoro & Bonarelli (1950). *Boll. Lab. chim. provinciali (Bologna)*, **1**, (2), 19. (427)
(373) Ambrosetti (1951). *Boll. Lab. chim. provinciali (Bologna)*, **2**, (1/2), 9. (428)
(374) Renko (1952). *Mondo Latte*, p. 711. (429)
(375) Kruisheer (1952). *Neth. Milk Dairy J.* **6**, 242. (430)
(376) Kruisheer (1951). *Nutr. Rev., N.Y.*, **9**, 118. (431)
(377) Lea (1952). *Chem. & Ind., Lond.*, no. 9, 178. (432)
(378) Raeithel (1952). *Z. LebensmittUntersuch.* **95**, 246. (433)
(379) Willstaedt & Reinart (1950). *Ark. Kemi*, **1**, 319. (434)
(380) Krukovsky (1952). *J. Dairy Sci.* **35**, 481. (435)
(381) Hvidsten, Mehlum & Simonsen (1952). *Meieriposten*, **41**, 241, 263. (436)
(382) Hvidsten, Mehlum & Simonsen (1952). *Meieriposten*, **41**, 145, 170, 186. (437)
(383) Honoré & Hvidsten (1952). *Meieriposten*, **41**, 309. (438)
(384) Pont (1952). *J. Dairy Res.* **19**, 316. (439)
(385) Krukovsky (1952). *J. Dairy Sci.* **35**, 21. (440)
(386) Forster & Sommer (1951). *J. Dairy Sci.* **34**, 992.
(387) Hunt & Hankison (1952). *Sth Dairy Prod. J.* **51**, 122. (441)
(388) Weinstein & Trout (1951). *J. Dairy Sci.* **34**, 554. (442)
(389) Weinstein & Trout (1951). *J. Dairy Sci.* **34**, 559, 565. (443)
(390) Weinstein (1951). *Milk Pl. Mon.* **40**, (11), 30. (444)
(391) Chilson, Martin & Whitnah (1950). *J. Dairy Sci.* **33**, 925. (445)
(392) Gelpi, Rusoff & Skole (1952). *J. Dairy Sci.* **35**, 93. (446)
(393) Smith, Loewnstein, Anderson & Olson (1952). *Milk Pl. Mon.* **41**, (5), 26. (447)
(394) Olson (1950). *Ice Cr. Rev.* **34**, (5), 94. (448)
(395) Keeney & Doan (1951). *J. Dairy Sci.* **34**, 713. (449)
(396) Holm, Wode & Thomé (1952). *Medd. Mejeriförsök.* no. 37. (450)
(397) Mattsson, Thomé & Swartling (1951). *Medd. Mejeriförsök.* no. 33. (451)
(398) Mukherjee & Goswami (1947). *J. Indian chem. Soc.* **24**, 239. (452)
(399) Mukherjee, Ray & Goswami (1950). *J. Indian chem. Soc.* **27**, 539, 545. (453)
(400) Mukherjee (1950). *J. Indian chem. Soc.* **27**, 557, 586, 589, 607, 613, 695, 699. (454)
(401) Martin & Julien (1951). *Québec Lait*, **10**, (7), 16. (455)
(402) Julien & Martin (1951). *Québec Lait*, **10**, (8), 16; (9), 14. (456)
(403) Julien & Martin (1952). *Québec Lait*, **11**, (3), 19. (457)
(404) Barnicoat (1950). *N.Z. J. Sci. Tech.* **32**, Sect. A, (2), 11.
(405) Barnicoat (1951). *N.Z. J. Sci. Tech.* **33**, Sect. A, (1), 37. (458)
(406) Mulder, Kruisheer, den Herder & van Ginkel (1949). *Neth. Milk Dairy J.* **3**, 37. (459)
(407) Ruuber (1951). *Mol. Prom.* **12**, (11), 20. (460)

(408) Coulter, Jenness & Crowe (1948). *J. Dairy Sci.* **31**, 986.
(409) Harland, Coulter & Jenness (1952). *J. Dairy Sci.* **35**, 643.
(410) White, Waite, Hawley, Clark & Henry (1952). *J. Dairy Res.* **19**, 339.
(411) Greenbank & Wright (1951). *J. Dairy Sci.* **34**, 815.
(412) Manus & Ashworth (1948). *J. Dairy Sci.* **31**, 517.
(413) Christensen, Decker & Ashworth (1951). *J. Dairy Sci.* **34**, 404.
(414) Decker, Ashworth & Christensen (1951). *J. Dairy Sci.* **34**, 412.
(415) Decker & Ashworth (1951). *J. Dairy Sci.* **34**, 633.
(416) Busch, Decker & Ashworth (1952). *J. Dairy Sci.* **35**, 524.
(417) Stull, Herreid & Tracy (1951). *J. Dairy Sci.* **34**, 80.
(418) Kägi (1950). *Mitt. Lebensm. Hyg., Bern*, **41**, 65.
(419) Kägi (1951). *Annu. agric. Suisse*, **52**, 803.
(420) Forss (1951). *Nature, Lond.*, **167**, 733.
(421) Olson, Beachboard, Anderson, Edmondson & Lacey. *J. Dairy Sci.* **35**, 485.
(422) Schwarzkopf (1950). *Amer. Milk Rev.* **12**, (8), 30.
(423) Forschner (1949). *Tejgazdaság*, **7**, 35.
(424) Forschner (1951). *Milk Dlr*, **40**, (7), 68.
(425) Csiszár, Tomka & Bittera (1949). *Tejgazdaság*, **7**, 129.
(426) Giolitti (1949). *Atti. Soc. ital. Sci. vet.* **3**, 543.
(427) Voiret (1951). *Ann. Falsif. Fraudes*, **44**, 284.
(428) Greene & Bell (1952). *Sci. Agric.* **32**, 619.
(429) Bindewald (1952). *Dtsch. med. Wschr.* **77**, 1015.
(430) Lämmler (1951). *Lebensmitteltierarzt*, **2**, (6), 86.
(431) British Standards Institution (1951). Stand. Specif. no. 1741.
(432) Schain (1950). *Milk Dlr*, **40**, (3), 49.
(433) Sager, Grant & Hammond (1951). *Conv. Proc. Milk Ind. Found.* **4**, 27.
(434) Tsvang & Litvinenko (1950). *Gigiena i Sanit.* **15**, (3), 51.
(435) Gentilini (1950). *Latte*, **24**, 219.
(436) D'yachenko, Kornilova & Vlodavets (1950). *Mol. Prom.* **11**, (8), 24.
(437) Brio & Vlodavets (1951). *Mol. Prom.* **12**, (7), 29.
(438) de Haan & Eisses (1950). *Neth. Milk Dairy J.* **4**, 225.
(439) Posthumus (1951). *Neth. Milk Dairy J.* **5**, 10.
(440) Scientific Committee for the Standardization of Methods for the Analysis of Milk and Dairy Products (1952). *Neth. Milk Dairy J.* **6**, 62.
(441) Hingst & Thomasow (1952). *Milchwissenschaft*, **7**, 16.
(442) Stoldt (1951). *Milchwissenschaft*, **6**, 152.
(443) Stoldt (1952). *Milchwissenschaft*, **7**, 232.
(444) Mohr & Koenen (1952). *Milchwissenschaft*, **7**, 120.
(445) Anselmi & Cesari (1952). *Mondo d. Latte* **1952**, 23.
(446) Herreid, Burgwald, Herrington & Jack (1952). *J. Ass. off. agric. Chem., Wash.*, **35**, 202.
(447) Sager (1952). *Chem. Engng News*, **30**, 4167.
(448) Wildasin & Anderson (1952). *Amer. Milk Rev.* **14**, (7), 56.
(449) Hem & Døvle (1950). *Norg. Landbrukshøgsk. Beretn. ForForsøk.* no. 67.
(450) Lehmann (1952). *Schweiz. Milchztg*, **78**, 122.
(451) Ducouret (1952). *Industr. lait.* no. 69, p. 133.
(452) Schulz, Effern & Roland (1952). *Milchwissenschaft*, **7**, 14.
(453) Henick (1951). *Food Tech., Champaign, Ill.*, **5**, 145.
(454) Dunkley (1951). *Food Tech., Champaign, Ill.*, **5**, 342.
(455) Patton & Kurtz (1951). *J. Dairy Sci.* **34**, 669.
(456) Dunkley & Jennings (1951). *J. Dairy Sci.* **34**, 1064.
(457) Kieferle, Feichtner & Pirner (1950). *Arb. Ber. Süddtsch. Vers.- u. Forsch. Milchw. Weihenstephan*, **1**, 37.
(458) Doan (1950). *Conv. Proc. Milk Ind. Found.* **4**, 22.
(459) Ystgaard, Homeyer & Bird (1951). *J. Dairy Sci.* **34**, 695.
(460) Babad & Grunpeter (1951). *J. Dairy Res.* **18**, 147.

(461) Tulchinskiy (1951). *Zh. Prikl. Khim. Leningr.* **24**, 179.
(462) Francis & Smith (1952). *J. Dairy Res.* **19**, 83.
(463) Balwant Rai Puri & Pritam Singh (1952). *Indian J. Dairy Sci.* **5**, 39.
(464) Mattson & Jensen (1950). *Analyt. Chem.* **22**, 182.
(465) Fairbridge, Willis & Booth (1951). *Biochem. J.* **49**, 423.
(466) Gorbach & Schäfer (1952). *Z. LebensmittUntersuch.* **95**, 168.
(467) Sarudi (1950). *Z. LebensmittUntersuch.* **91**, 342.
(468) Potter (1950). *J. Dairy Sci.* **33**, 803.
(469) Larsen & Gould (1951). *J. Dairy Sci.* **34**, 16.
(470) Stewart (1951). *J. Dairy Sci.* **34**, 743.
(471) Larson & Jenness (1950). *J. Dairy Sci.* **33**, 896.
(472) Slavyanov & Titov (1951). *Mol. Prom.* **12**, (1), 35.
(473) Balwant Rai Puri & Pritam Singh (1951). *Indian J. Dairy Sci.* **4**, 135.
(474) Pedersen (1951). *Nord. Mejeri-Tidsskr.* **17**, 124.
(475) Dumazert & Bozzi-Tichadou (1952). *C.R. Soc. Biol., Paris*, **146**, 468.
(476) Shahani & Sommer (1951). *J. Dairy Sci.* **34**, 1003.
(477) Ashworth & Drueger (1951). *Proc. W. Div. Amer. Dairy Sci. Ass.* p. 74.
(478) Spies & Chambers (1949). *Analyt. Chem.* **21**, 1249.
(479) Portner & Högl (1952). *Mitt. Lebensm. Hyg., Bern*, **43**, 505.
(480) Spies & Chambers (1951). *J. biol. Chem.* **191**, 787.
(481) Block & Bolling (1951). *The Amino-acid Composition of Proteins and Foods. Analytical Methods and Results.* Springfield, Illinois: Charles C. Thomas.
(482) Thomasow (1951). *Kieler Milchwirtschaft. ForschBerichte*, **3**, 419.
(483) Johnson & King (1951). *Analyst*, **76**, 504.
(484) Bakalor (1951). *Fmg S. Afr.* **26**, 355.
(485) Musso (1950). *Mondo Latte*, p. 289.
(486) Mitchell (1951). *J. Ass. off. agric. Chem., Wash.*, **34**, 252.
(487) Lang & Pfleger (1951). *Mikrochemie*, **36/37**, 1174.
(488) Paulsen (1951). *Kieler Milchwirtschaft. ForschBerichte*, **3**, 131.
(489) McAllister (1951). *Analyst*, **76**, 238.
(490) Neidlinger, Kempf & Stewart (1952). *J. Dairy Sci.* **35**, 305.
(491) Tiersma & Venekamp (1950). *Neth. Milk Dairy J.* **4**, 158.
(492) Venekamp & Kruisheer (1950). *Neth. Milk Dairy J.* **4**, 165.
(493) Ling (1951). *J. Sci. Food Agric.* **2**, 279.
(494) Sarudi (1952). *Z. LebensmittUntersuch.* **94**, 97.
(495) Arakawa (1950). *Tohoku J. exp. Med.* **53**, 167.
(496) Thin & Robertson (1952). *Biochem. J.* **51**, 218.
(497) Wokes, Wedgwood & Wyatt (1952). *Biochem. J.* **50**, xix.
(498) Borrell (1950). *An. Bromatol., Madrid*, **1**, 455.
(499) Buruiana & Niculesco (1951). *Lait*, **31**, 481.
(500) Jacquet & Nir (1952). *Ann. Falsif. Fraudes*, **45**, 21.
(501) Pezzani (1948). *Rev. Fac. Cienc. quim. La Plata*, **23**, 171.
(502) Hänni (1951). *Mitt. Lebensm. Hyg., Bern*, **42**, 114.
(503) Schwarz & Krauss (1952). *Kieler Milchwirtschaft. ForschBerichte*, **4**, 579.
(504) Schober & Fricker (1952). *Z. LebensmittUntersuch.* **95**, 107.
(505) Smeets & Seekles (1952). *Nature, Lond.*, **169**, 802.
(506) Hunter (1950). *Analyst*, **75**, 91.
(507) Pomorska (1950). *Roczn. Zakl. Hig., Warsz.*, **1**, 215.
(508) Streichówna (1950). *Roczn. Zakl. Hig., Warsz.*, **1**, 295.
(509) Brown (1952). *Biochem. J.* **51**, 237.
(510) Sternberg, Colas & Kahn (1951). *Mikrochemie*, **36/37**, 942.
(511) Müller (1951). *Mitt. Lebensm. Hyg., Bern.*, **42**, 532.
(512) Gerber & Siegenthaler (1952). *Schweiz. Milchztg*, **78**, 133.
(513) Gerber & Siegenthaler (1953). *Schweiz. Milchztg*, **79**, 6.
(514) Scott (1951). *J. Ass. off. agric. Chem., Wash.*, **34**, 350.

(515) Pien, Desirant & Rochelle (1951). *Ann. Falsif. Fraudes*, **44**, 290.
(516) Claborn & Beckman (1952). *Analyt. Chem.* **24**, 220.
(517) Tufts, Darling & Kimball (1950). *J. Ass. off. agric. Chem., Wash.*, **33**, 976.
(518) Stoltz & Hankison (1950). *Amer. Milk Rev.* **12**, (12), 60.
(519) Churchill, Drury, Lewis, Frank & Bryan (1951). *Milk Pl. Mon.* **40**, (4), 28.
(520) British Standards Institution (1952). Stand. Specif. no. 769.
(521) Weber (1951). *J. Ass. off. agric. Chem., Wash.*, **34**, 243.
(522) Weber & Edelson (1952). *J. Ass. off. agric. Chem., Wash.*, **35**, 194.
(523) Jax (1952). *Öst. Milchw.* **7**, (4), 48.
(524) Overbeck, Mossel, Mossel-Hens & Reith (1951). *Rec. Trav. chim. Pays-Bas*, **70**, 63, 297, 331.
(525) Roeder (1951). *Molkereiztg, Hildesh*, **5**, 691, 1241.
(526) Vlodavets & Berliner (1951). *Mol. Prom.* **12**, (6), 21.
(527) Muller (1952). *Aust. J. Dairy Tech.* **7**, (2), 44.
(528) Dudenkov (1951). *Mol. Prom.* **12**, (3), 36.
(529) Nijkamp (1951). *Anal. chim. Acta*, **5**, 325.
(530) Gerritsma (1952). *Neth. Milk Dairy J.* **6**, 266.
(531) Hillig & Ahlmann (1951). *J. Ass. off. agric. Chem., Wash.*, **34**, 777.
(532) Hillig (1951). *J. Ass. off. agric. Chem., Wash.*, **34**, 782.
(533) Hillig (1952). *J. Ass. off. agric. Chem., Wash.*, **35**, 748.
(534) Armstrong, Harper & Gould (1951). *Butt. Cheese Milk Prod. J.* **42**, (5), 28.
(535) Armstrong & Harper (1952). *J. Dairy Sci.* **35**, 59, 342.
(536) Schaffer & Holm (1950). *J. Dairy Sci.* **33**, 865.
(537) Hartman & White (1952). *Analyt. Chem.* **24**, 527.
(538) Miller & Tracy (1952). *J. Dairy Sci.* **35**, 292.
(539) Thaler & Scheler (1951). *Z. LebensmittUntersuch.* **93**, 220.
(540) Thaler & Scheler (1951). *Z. LebensmittUntersuch.* **93**, 286.
(541) Thaler & Scheler (1952). *Z. LebensmittUntersuch.* **95**, 1.
(542) Diemar & Janecke (1950). *Dtsch. Lebensmitt. Rdsch.* **46**, 110.
(543) British Standards Institution (1952). Stand. Specif. no. 770.
(544) Perlmutter & Horwitz (1952). *J. Ass. off. agric. Chem., Wash.*, **35**, 204.
(545) Larsson (1952). *Mejeritek. Medd.* **13**, 73.
(546) Cook, Follstad & Fisher (1953). *J. Ass. off. agric. Chem., Wash.*, **36**, 132.
(547) Ozimov, Guygo & Alyamovskiy (1952). *Mol. Prom.* **13**, (12), 37.
(548) Lodin & Buhrgard (1952). *Svenska Mejeritidn.* **44**, 405.
(549) Schwarz, Mumm & Neumann (1949). *Süddtsch. Molkereiztg*, **70**, 1383.
(550) Kaloyereas (1950). *Olii min.* **27**, (6/7), 73.
(551) Buchholz & Smith (1953). *Z. LebensmittUntersuch.* **96**, 245.
(552) Harper (1952). *J. Dairy Sci.* **35**, 479.
(553) Storgårds & Lindqvist (1952). *Svenska Mejeritidn.* **44**, 103.
(554) Schulz & Mrowetz (1952). *Dtsch. Molkereiztg*, **73**, 495, 530.
(555) Shakhbazyan (1951). *Mol. Prom.* **12**, (10), 25.
(556) Choi, Tatter & O'Malley (1951). *J. Dairy Sci.* **34**, 845.
(557) Choi, Tatter & O'Malley (1951). *Analyt. Chem.* **23**, 888.
(558) Mohr & Koenen (1951). *Kieler Milchwirtschaft. ForschBerichte*, **3**, 665.
(559) Brouwer (1952). *Neth. Milk Dairy J.* **6**, 53.
(560) Strobel & Babcock (1951). *J. Dairy Sci.* **34**, 167.
(561) Vuillaume (1952). *Lait*, **32**, 609.
(562) Blanchard (1951). *Lait*, **31**, 609.
(563) Talley & Hunter (1952). *J. Amer. chem. Soc.* **74**, 2789.
(564) British Standards Institution (1951). Stand. Specif. no. 1742.
(565) British Standards Institution (1951). Stand. Specif. no. 1743.
(566) Khlopin, Litvinova & Privalova (1952). *Gigiena i Sanit.* (4), p. 47.
(567) Blaxter & Allcroft (1950). *J. comp. Path.* **60**, 133.
(568) Blaxter (1950). *J. comp. Path.* **60**, 140.
(569) Meinsma (1951). *Chem. Weekbl.* **47**, 85.

(570) Devender, Lyon & Bird (1952). *J. Dairy Sci.* **35**, 116.
(571) Bergman (1947). *J. Ass. off. agric. Chem., Wash.*, **30**, 613.
(572) Bureau of Dairy Industry (1953). *Agric. Handb. U.S. Dep. Agric.* **51**.
(573) Horwitz (1951). *J. Ass. off. agric. Chem., Wash.*, **34**, 236.
(574) Scientific Committee for the Standardization of Methods for the Analysis of Milk and Dairy Products (1952). *Neth. Milk Dairy J.* **6**, 180, 184.
(575) Zijlstra, Leuver, Kruisheer & Vester (1951). *Neth. Milk Dairy J.* **5**, 51.
(576) Indian Council of Agricultural Research (1951). *Report.* Allahabad, U.P., India: Allahabad Agricultural Institute.
(577) Kothavalla (1951). *Bull. Indian Coun. agric. Res.* **70**.

## REFERENCES ARRANGED ALPHABETICALLY

Aas (36); Achaya & Hilditch (107); Adam (73); Alekseeva & Kasatochkin (351); Alison (368); Ambrosetti (373); Anselmi & Cesari (445); Antoine (23); Antoniani & Cerutti (302); Antoniani *et al.* (301); Arakawa (495); Archibald (55); Armstrong & Harper (535); Armstrong *et al.* (534); Arrington & Krienke (353); Asdrubali (59); Ashworth (293); Ashworth & Drueger (477); Aschaffenburg (146, 148); Aschaffenburg & Rowland (1); Aschaffenburg *et al.* (283).

Babad & Grunpeter (460); Babcock *et al.* (305); Bailey (14, 15); Bakalor (40, 41, 42, 484); Balch *et al.* (13); Balwant Rai Puri & Pritam Singh (463, 473); Barnabel & Mari (131); Barnicoat (404, 405); Barry (217); Bartlett & Kay (11); Bartlett *et al.* (53, 54); Bartley *et al.* (108); Baud *et al.* (172); Beacom & Bowland (71); Beck (155); Bell & Mucha (307, 311); Bena-Medean & Liteanu (366); Bergman, R.E. (571); Bergman, T. & Joost (119); Bergum (350); Berridge (177, 178); Bessey & Love (147); Bindewald (429); Blanchard (562); Blaxter (568); Blaxter & Allcroft (567); Block (125); Block & Bolling (481); Bosticco *et al.* (4, 5, 6); Borrell (498); Breirem *et al.* (60); Brintzinger & Wolff (236); Brio & Vlodavets (437); Brouwer (559); Brown (509); Brunner (343); Buchholz & Smith (551); Burbelo (191); Burgwald (149); Buruiana (194); Buruiana & Geraniou (195); Buruiana & Niculesco (499); Busch *et al.* (416).

Cacioppo (317); Campbell & Work (219); Camus & Alifax (162); Carbone (45); Casini (56); Cerutti (117, 304); Chance (190); Chanda & Owen (137, 140); Chanda *et al.* (138, 139); Cherbuliez & Baudet (229, 230); Chilson *et al.* (391); Choi *et al.* (556, 557); Christensen *et al.* (413); Churchill *et al.* (519); Claborn & Beckman (516); Cook, G. T. & Hughes (144); Cook, J. H. *et al.* (546); Coulson & Stevens (261); Coulter *et al.* (408); Csiszár *et al.* (425).

Davidov (300); Davidov & Barabanshchikov (313); Davis (12); Dawson (269); Decker & Ashworth (415); Decker *et al.* (414); De Haan & Eisses (438); Devender *et al.* (570); Deysher & Webb (348); Dharamarajan *et al.* (354); Dicks *et al.* (26); Diemair & Janecke (542); Doan (309, 458); Dovey & Campbell (232); Ducouret (451); Dudenkov (528); Dumazert & Bozzi-Tichadou (475); Dunkley (454); Dunkley & Jennings (456); D'yachenko (174); D'yachenko & Vlodavets (223); D'yachenko *et al.* (436).

Edwards (17); Ellenberger *et al.* (50); El-Sokkary *et al.* (72); Ecenhuis (77).

Fabris (44, 58); Fagen & Hussong (87); Fagen *et al.* (83); Fairbridge *et al.* (465); Farfaletti-Casali & Cerutti (303); Featherstone *et al.* (18); Fialkov (288); Forschner (423); Forss (420); Forster & Sommer (386); Foster *et al.* (284); Francis & Smith (462); Fraser & Powell (246).

Gelpi *et al.* (392); Gentilini (435); Gerber & Siegenthaler (512, 513); Gerritsma (530); Giolitti (426); Goded y Mur (365); Gonashvili (183); Gorbach & Schäfer (466); Gordon & Semmett (262); Grant (312); Greenbank (104); Greenbank & Wright (411); Greene & Bell (428); Griebel & Schwarz (347); Groves *et al.* (275, 277); Gulvady *et al.* (37).

Hadland & Solberg (370); Håkansson & Sjöström (163); Haller & Bell (306); Halwer *et al.* (271); Hannan & Lea (340); Hänni (502); Hansen, R. P. & Shorland (113, 114); Hansen, R. P. *et al.* (112); Hansen, S. A. *et al.* (142); Hansson (320); Hare *et al.* (220); Harland *et al.* (265, 409); Harper (97, 552); Harper & Gould (96); Harris & Hellerman (199); Hartman & White (537); Heidebrecht *et al.* (68); Heinemann & Parker (268); Hellbrügge (259); Hem & Døvle (449); Henick (453); Herreid *et al.* (446); Herrington (184); Heyndrickx & de Vleeschauwer (214, 215, 216); Higgins & Hayes (235); Higgins *et al.* (241); Hilditch (109); Hillig (532, 533); Hillig & Ahlmann (531); Hillig *et al.* (78); Hingst & Thomasow (441); Hipp *et al.* (226, 227); Hiscox *et al.* (89); Hodson (126); Hoffmann (362); Hofstee (196); Hollender (346); Holm *et al.* (396); Hoover (244); Honoré & Hvidsten (383); Horwitz (573); Hostettler & Imhof

(170); Hostettler & Rüegger (169); Hove & Harris (116); Hung-Chun Kung *et al.* (208); Hunt & Hankison (387); Hunter (506); Hutton & Patton (266); Hvidsten *et al.* (381, 382).

Irvine (81); Isidoro & Bonarelli (372); Isidoro & Pavolini (371).

Jack *et al.* (47); Jacobsen (273); Jacquet & Nir (500); Jacquet & Saingt (164); Janzen & Swanson (345); Jarrousse *et al.* (157, 158); Jax (523); Johansson (28); Johansson *et al.* (27); Johnson, E. I. & King (483); Johnson, B. C. & Swanson (267); Julien & Martin (402, 403).

Kägi (418, 419); Kalckar *et al.* (197); Kaloyereas (550); Kamal & Turner (233); Kannan & Basu (141, 185, 209); Karambelkar *et al.* (250); Kastens & Baldauski (252); Keeney & Doan (395); Kermack & Miller (322); Khambatta & Dastur (38); Khlopin *et al.* (566); Kieferle & Seuss (152); Kieferle *et al.* (457); Kielbasińska (22); Kiermeier (237); King (294, 295, 296); Kofiányi (122); Korkman (29); Kosikowsky (85, 86); Kothavalla (577); Krauss (321); Krett *et al.* (90); Krishnamurti & Subrahmanyan (182); Kruisheer (375); Krukovsky (380, 385); Krukovsky & Loosli (33); Kublitz (20).

Lämmler (430); Lang & Pfleger (487); Larsen & Gould (469); Larson & Jenness (279, 471); Larsson (545); Laskowski & Laskowski (205); Laskowski *et al.* (206); Lawrence (349); Lea (342, 377); Lea & Hannan (335, 336, 337); Lea & Rhodes (339); Lea *et al.* (128, 338); Lear & Foster (143); Lehmann (450); Lemacher *et al.* (204); Lembke & Christiansen (207); Lengemann *et al.* (57); Lewis & Lea (341); Ling (493); Lodin & Buhrgard (548); Loncin & Jacqmain (188); Lonka (24); Lythgoe (357, 358); Lubert *et al.* (186, 187); Lust (239).

Mahadevan (16); Mahajan & Mathur (364); Malyoth *et al.* (32); Mantillaro *et al.* (3); Manus & Ashworth (412); Martin & Julien (401); Mattenheimer *et al.* (167); Mattick (91); Mattson & Jensen (464); Mattsson *et al.* (118, 397); Maxa (101); McAllister (489); McClymont (105); McDougall (281); McDowall (82); McDowell (115); McFarren & Mills (280); McGlasson & Boyd (352); McIntyre *et al.* (285); McKerns (173); McLean (19); McMeekin & Marshall (225); McMeekin *et al.* (224, 276); Meinsma (569); Mellon *et al.* 243, 245); Miller, D. E. & Tracy (538); Miller, R. A. (323); Miller, R. A. & Jackson (324); Mitchell (486); Mohr & Corte (325, 326); Mohr & Kaufmann (297); Mohr & Koenen (444, 558); Moody *et al.* (2); Morell (198); Morgan *et al.* (79); Morice (120); Morton (132); Moster & Chapman (334); Mucha & Bell (308); Mukherjee (400); Mukherjee & Goswami (398); Mukherjee *et al.* (399); Mulder *et al.* (406); Müller, E. (511); Muller, L. L. (527); Munch-Petersen (51); Murthy *et al.* (106); Musso (485).

Neidlinger *et al.* (490); Neseni & Freimuth (63, 67); Nichols & Few (21); Nickerson *et al.* (299); Nijkamp (529); Nitschmann (221); Nitschmann & Varin (166); Nitschmann & Zahler (168); Nitschmann & Zürcher (231).

Odenwald (287); Olson (394); Olson *et al.* (421); Orlova (92); Ormsbee (34); Ovchinnikov (238); Overbeck *et al.* (524); Ozimov *et al.* (547).

Paley & Tzall (359); Panasenkov (344); Parisi (43); Paschke (210); Patton (103, 331, 332); Patton & Josephson (329); Patton & Kurtz (455); Patow (31); Paulsen (488); Paulson *et al.* (249); Pedersen, A. H. (251); Pedersen, J. W. & Baker (248); Pedersen, N. T. (474); Pereira de Matos & Vieira de Sá (181); Perlmann (247); Perlmutter & Horwitz (544); Pezzani (501); Pien *et al.* (515); Piskarev & Kremnev (292); Plomley *et al.* (240); Polis & Shmukler (189, 203); Polis *et al.* (260, 274); Polonovski & Robert (129, 130); Polonovski *et al.* (200, 201, 202); Pomorska (507); Pont (384); Pope & Roy (8); Portner & Högl (479). Posthumus (159, 439); Potter (468); Princivalle (66); Provan (9).

Raeithel (378); Ramakrishnan & Banerjee (315, 316); Randoin & Jourdan-Vatinel (94, 95); Rangappa & Banerjee (289); Rees (356); Renko (374); Riedel (328); Riley (270); Rishoi (298); Ritland *et al.* (272); Roeder (318, 319, 525); Rothman (360); Rowallan (52); Rowland (10); Rowland *et al.* (7); Rudowska *et al.* (102); Ruuber (407).

Sager, C. A. (363); Sager, O. S. (447); Sager, O. S. *et al.* (433); Salati (180); Sarudi (467, 494); Schäfer (257); Schaffer (327); Schaffer & Holm (536); Schain (432); Schmid, A. & Zollikofer (35); Schmid, H. (286); Schober & Fricker (504); Schormüller (127); Schulz (175); Schulz & Mrowetz (554); Schulz & Sydow (193); Schulz *et al.* (80, 452); Schwander *et al.* (165); Schwarz (150); Schwarz & Effern (369); Schwarz & Krauss (503); Schwarz & Lange (151, 153); Schwarz & Mumm (98); Schwarz & Thomasow (99); Schwarz *et al.* (100, 549); Schwarzkopf (422); Scott (514); Seelemann (156); Seidler (64, 65); Shahani & Sommer (123, 476); Shakhbazyan (555); Sheffey *et al.* (69, 121); Shorland (110); Shorland & Johannesson (111); Shvabe (46); Simonart & Mayaudon (253); Simskaya (160, 161); Smeets & Seekles (505); Smith, A. C. *et al.* (393); Smith, D. M. (70); Smith, E. L. & Brown (282); Slatter & van Winkle (213); Spies & Chambers (478, 480); Slavyanov & Titov (472); Springer & Quentin (124); Stanley, *et al.* (258); Sternberg *et al.* (510); Stewart (470); Storgårds (255); Storgårds & Lindqvist (254, 553); Stoldt (367, 442, 443); Stoltz & Hankin-

son (518); Streichówna (508); Strobel & Babcock (560); Strogyll *et al.* (330); Stüber & Göbelt (25); Stull *et al.* (417); Szkilladziowa *et al.* (93).

Talley & Hunter (563); Tammisto & Tuomainen (76); Tarassuk & Simonson (333); Ter Horst (234); Thaler & Scheler (539, 540, 541); Thin & Robertson (496); Thomasow (482); Thomsen (74); Tiersma & Venekamp (491); Titov (179); Tobias *et al.* (211, 212); Trautman *et al.* (84); Tsvang & Litvinenko (434); Tufts *et al.* (517); Tulchinskiy (461); Tuomainen (75).

Uesaka & Tze-Yee Chang (49); Uesaka *et al.* (48); Ulvesli *et al.* (62); Uuspää & Torsti (88).

Valen & Valen (61); Van der Smissen (192); Varin (171); Venekamp & Kruisheer (492); Venkatappaiah & Basu (39); Venkateswara Rao *et al.* (355); Vereshchagina (30); Vlodavets & Berliner (526); Vlodavets *et al.* (222); Voiret (427); Vuillaume (561).

Wagener & Eberhardt (154); Weber (521); Weber & Edelson (522); Wegelin (256); Weil & Buchert (278); Weinstein (390); Weinstein & Trout (388, 389); Weinstein *et al.* (263, 264); White *et al.* (410); Whittlestone (290, 291); Wight & Tramer (145); Wildasin & Anderson (448); Wildasin & Doan (310); Willstaedt & Reinart (379); Wokes *et al.* (497); Work *et al.* (218); Wormell (242).

Ystgaard *et al.* (361, 459).

Zijlstra *et al.* (575); Zimmerman (176); Zittle & DellaMonica (133, 134, 135, 136); Zweig & Block (228).

637.0:53

## PART II. RHEOLOGY AND GENERAL PHYSICS

### General

Several books and monographs, wholly or partly concerned with the rheology of dairy products, have been published during the period under review. Miss Baron(1) summarizes work on 'the mechanical properties of cheese and butter'; and, writing for rather different readers, Baron & Scott Blair have contributed a chapter on 'rheology of cheese and curd' in an International Rheology Monograph dealing with foodstuffs in general(2). The same monograph contains chapters on 'milk, cream, ice-cream mixes and similar products' by Scott Blair, on 'the consistency of butter' by Mulder, and on 'psycho-rheology of foodstuffs' by Harper. This last author has also published a monograph entitled 'Psychological and psychophysical studies of craftsmanship in dairying'(3), which is mainly concerned with his own researches while at the National Institute for Research in Dairying.

In Mrak & Stewart's *Advances in Food Research*, III(4), there are two chapters which have some bearing on dairy physics. Coulter, Jenness & Geddes have contributed a fifty-page review of work on dried milk products; but the inclusion in the title of 'physical aspects' refers rather to the general physical condition of the product than to measurements of any specific physical properties. A review by Proctor & Goldblith of work on electromagnetic radiations in food technology as a whole has some bearing on dairy problems, though the interest is rather from the engineering than from the physical standpoint. The chapter is marred by an unfortunate confusion between sonic and ultrasonic waves on the one hand and electromagnetic radiations of various frequencies on the other. No doubt the authors are clear as to which remarks refer to which type of radiation, but it is almost impossible for the reader to avoid confusion.

### Physical properties of liquid milk

Whittlestone(5) has measured a number of physical properties of sow's milk as a function of the stage of lactation. With the exception of pH, which increases during the lactation period, the results are somewhat inconclusive. Fat percentage tends to fall, with a corresponding rise in electrical conductivity and density, and a fall in viscosity (as measured in an Ostwald viscometer). In the second part of the work, the author studies the distribution of size of fat globules. (See Section on Physical Chemistry.)

D'yachenko(6) has contributed a review article on chemical and physical properties of milk. His main interest is in colloidal properties, but measurements of colour of butterfat and X-ray diagrams of lactose are discussed.

Goded y Mur(7) has measured surface tension and viscosity of large numbers of milk samples and claims that additions of >10% water can be detected by deviations from his mode* values, which are 50·4 dynes/cm. and 2·04 cp. respectively. (The last figure is of little value since temperature is not quoted.) Venkatasubramanian & Ramakrishnan(8) have measured refractive index and fluorescence of liquid milk. (Articles on the use of the electron microscope in relation to milk proteins are reviewed in the section on Physical Chemistry.)

It is remarkable that so few accurate measurements of the physical properties of milk are to be found in the literature. This is especially true of physical changes during modern processes of pasteurization. Summarizing a discussion at the XIIIth International Dairy Congress (1953) on 'Changes which milk undergoes as a result of heating', Sjöström(9) wisely remarks: 'Laboratory investigations must be supplemented with work on the heating methods used in the dairy industry. We need also more precise methods for determining the rapid temperature changes of the milk in the modern H.T.S.T. treatment.'

## Butter

King(10,11) has contributed two review articles on continuous butter-making and on theories of churning to which the reader is referred.

Prentice(12) describes techniques for measuring spreading properties of butter, and studies changes in rheological properties with age. He reports also(13) that a high electrical conductivity is often associated with rapid deterioration, doubtless due to the detrimental effects of a continuous aqueous phase through the emulsion. The same author describes applications of rheological methods to margarine and compound cooking fats(14) and the potentialities of multiple-factor analysis to elucidate the significance of the data so obtained are discussed.

Somewhat similar rheological methods for testing butter (the bending beam, rupture tests, sectility, etc.) are described by Hofmann, Mosimann & Oesch(15).

Certain Russian authors have studied the working of butter. Thus Shuvalov(16) churns butter with a stirrer and records the power consumption with time in relation to the fullness of the churn. He reports a sharp break in the curve at the moment of butter formation and plots logarithmic time needed for churning against the fullness of the churn and the weight of the resulting butter. Gordenko(17) is concerned with heat dissipation in churning between concentric cylinders for which he finds an optimum clearance. The time required for the butter to form passes through a minimum as clearance is increased. Chekulaev(18) describes an apparatus of the balance type for measuring shear-resistance and gives curves relating this property to temperature for butters at different seasons of the year and in relation to cow rations.

Certain workers have studied the absorption spectra of butter fats, especially in the ultra-violet region. Lembke, Kaufmann & Farfaletti-Casali(19) find peaks around 230, 268 and 302 mμ, which they ascribe to conjugated dienes, trienes and tetraenes respectively. The dienes and trienes give triple peaks. Quite drastic treatment of cream and butter failed to alter the spectra. Margarine fats show characteristically different spectra and the method can be used to detect adulteration of butter with vegetable fats.

## Cheese

Comparatively few new experiments have been reported during the period under review.

Wearmouth(20,21) has studied the hardness ('firmness') of Cheddar and Cheshire cheese by means of a sphere indentor, in relation to changes in temperature. A triple needle penetrometer is also described. Preliminary experiments suggest that indentor firmness is linearly related to Fahrenheit temperature over a considerable range.

On the psychological aspects of craftsmanship in cheese-making and judging, Harper(22) and Sheppard(23,24) have contributed papers of general interest. The latter has also discussed(25) the importance of well-established psychophysical errors in subjective judgments of cheese firmness. The most important practical finding is that the firmness of any particular cheese tends to be

* *Medio* is taken to mean 'mode' rather than 'median'. It is certainly not the 'mean'.

judged in relation to the average firmness of the group to which it belongs. Thus a cheese is given a different rating according to its position in the series being judged. A cheese presented immediately after a firm cheese has been judged, tends to be assessed as softer than when given following a soft cheese.

The importance of the feel of the surface of materials in judging firmness is stressed in a paper by Sheppard & Scott Blair(26).

### Cervical secretions

Scott Blair has contributed a chapter on rheology of secretions to a book by Frey-Wyssling(27) on rheological problems in biology, and summarizes work to date (1952). He also discussed recent developments comparing man and the bovine, at a meeting of endocrinologists and gynaecologists(28). Glover & Scott Blair(29) described further progress in the work discussed in the last Review. In an experiment later shown to have involved too frequent sampling for optimum discrimination, secretions from some 80% of normal pregnant cows examined showed significant changes in the rheology of their secretions after 5 weeks' pregnancy. Subfertility was reflected in some cases by abnormally high, and in some cases by abnormally low values of the property measured. In the latter case, pregnancy tended to terminate in abortion.

Though concerned with human rather than bovine artificial insemination, a paper by Cohen, Stein & Kaye(30) deserves mention as a warning against confusing quite distinct rheological properties such as *Spinnbarkeit* and viscosity, which are only correlated under special conditions. The authors do, however, confirm early findings that high *Spinnbarkeit* is a characteristic of cervical secretions at about the time of ovulation.

Glover(31) has recorded characteristic differences in ultra-violet absorption spectra and, more tentatively, in flow-birefringence in diluted secretions from pregnant, dioestrous and oestrous conditions in the cow. These may well be connected with the rheological differences already discussed and perhaps also with van Klinkenberg's(32) recent observation that there is a marked rise in alkaline phosphatase activity in secretions just before oestrus with some evidence of smaller increases some 8–12 days post oestrum.

### Miscellaneous

Pette & Lolkema(33) measure the consistency of milk-yogourt in terms of the rate of fall of a partly counterposed steel ball, the acidity and temperature (18° C.) being controlled. The consistency is affected by the stage in lactation and by the solids-not-fat of the milk, which is adjusted by addition of milk powder. The 'individual cow' factor is also significant. Homogenization increases the consistency. There is no close correlation between consistency and whey separation. This latter is a serious problem, partly controlled by additions on calcium chloride.

Though on the borderline of the subject-matter of this Section, mention should be made of Sheppard's(34) work on the measurement of the mammary glands of young heifers by palpation. A comparison with objective standards, using a dummy-udder, showed that the inaccuracies of the method of palpation can sometimes be considerably reduced by measuring and correcting for systematic individual errors.

National Institute for Research in Dairying, University of Reading

G. W. Scott Blair

### REFERENCES

(1) Baron (1952). *The Mechanical Properties of Cheese and Butter*. London: Dairy Industries.
(2) Scott Blair (Ed.) (1953*a*). *Foodstuffs, their Plasticity, Fluidity and Consistency*. Amsterdam: N. Holland Publ. Co.
(3) Harper (1952*a*). Psychological and psychophysical studies of craftsmanship in dairying. *Brit. J. Psychol. Monogr. Suppl.* no. 28.
(4) Mrak & Stewart (1951). *Advances in Food Research*, III. New York: Academic Press Inc.
(5) Whittlestone (1952). *J. Dairy Res.* **19**, 330, 335.

(6) D'YACHENKO (1952). *Mol. Prom.* **13**, 23.
(7) GODED Y MUR (1951). *An. Bromatol.* **3**, 267.
(8) VENKATASUBRAMANIAN & RAMAKRISHNAN (1952). *Science & Culture*, **17**, 260.
(9) SJÖSTRÖM (1953). *Proc. XIIIth Int. Dairy Congr. Hague*, **1**, 357.
(10) KING (1952). *Dairy Sci. Abstr.* **14**, 226.
(11) KING (1953). *Dairy Sci. Abstr.* **15**, 590.
(12) PRENTICE (1953*a*). *Proc. XIIIth Int. Dairy Congr. Hague*, **2**, 723.
(13) PRENTICE (1953*b*). *Proc. XIIIth Int. Dairy Congr. Hague*, **2**, 727.
(14) PRENTICE (1952). *B.F.M.I.R.A. Res. Rep.* no. 37 (released).
(15) HOFMANN, MOSIMANN & OESCH (1953). *Schweiz. Milchztg*, **79**, 29.
(16) SHUVALOV (1951). *Mol. Prom.* **5**, 29.
(17) GORDENKO (1952). *Mol. Prom.* **13**, 30.
(18) CHEKULAEV (1953). *Mol. Prom.* **14**, 32.
(19) LEMBKE, KAUFMANN & FARFALETTI-CASALI (1953). *Milchwissenschaft*, **8**, 118.
(20) WEARMOUTH (1952). *Dairy Ind.* **17**, 994.
(21) WEARMOUTH (1953). *Proc. XIIIth Int. Dairy Congr. Hague*, **2**, 583.
(22) HARPER (1952*b*). *Dairy Ind.* **17**, 236.
(23) SHEPPARD (1952*a*). *Dairy Ind.* **17**, 707.
(24) SHEPPARD (1953*a*). *Food*, **22**, 13.
(25) SHEPPARD (1952*b*). *J. Dairy Res.* **19**, 348.
(26) SHEPPARD & SCOTT BLAIR (1952). *Quart. J. exp. Psychol.* **4**, 101.
(27) FREY-WYSSLING (1952). *Problems of Flow in Biology*. Amsterdam: N. Holland Publ. Co.
(28) SCOTT BLAIR (1953*b*). *J. Endocrin.* **9** (i).
(29) GLOVER & SCOTT BLAIR (1953). *J. Endocrin.* **9**, 160.
(30) COHEN, STEIN & KAYE (1952). *Fertility and Sterility*, **3**, 201.
(31) GLOVER (1953). *Nature, Lond.*, **172**, 255.
(32) VAN KLINKENBERG (1953). *Nature, Lond.*, **172**, 397.
(33) PETTE & LOLKEMA (1951). *Neth. Milk Dairy J.* **5**, 27.
(34) SHEPPARD (1953*b*). *J. Dairy Res.* **20**, 127.